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UNCSTD was a modest success

Developing countries have been told to take science and technology much more seriously — and have been given a little help to do so. Much depends on how they use the first \$250 million.

As the couple of thousand delegates who have attended the UN Conference on Science and Technology for Development (UNCSTD) in Vienna disperse, most will be asking themselves whether the conference was a success or a failure. For some, who came with very specific objectives such as to do business with other delegates, the answer will be equally specific. But for the vast majority who came with rather more diffuse expectations the answer will be complex. In the end, of course, no real assessment can be made until we can apply a historian's eye to it and ask whether the whole range of activities associated with UNCSTD — the national discussions, the regional conferences, the involvement of non-governmental organisations (NGOs), the pre-conference negotiations quite apart from UNCSTD itself — has significantly quickened the pace of development. And for that the historian must be sure that what 'development' means is how people themselves want to share in the intellectual and physical resources of the world. This means freedom not just for developing countries to design their own development strategies, free from the dictates of the developed world and its technology-salesmen. It also means freedom for the people of developing countries to choose not necessarily to go along with the development strategy worked out for them by a Westernised elite in their capital city. It means freedom for individuals to go their own separate ways in pursuit of individual development. So the influence of UNCSTD is hardly to be measured in a short space of time by adding together a few numbers.

In tangible terms, however, UNCSTD will leave behind a mountain of national reports, regional summaries, discussion papers — and a handful of documents debated at the conference itself. We report on subsequent pages on how these documents (the Programme of Action and a paper on science, technology, development and the future) were negotiated over the two weeks. Since the UN rule is to proceed by consensus if at all possible, they are necessarily broad generalisations and in many cases contain exhortations that individual countries will simply be unable to act upon. Negotiation is carried out by blocs and the interests of individual nations sometimes have to be surrendered for reasons of solidarity. Most publicity at the conference centred on the difficulty that the European Economic Community had in keeping to a unified position, particularly on questions of new

financial support. But there is no reason to suppose that the Group of 77 (the developing nations), for which Tunisia acted, was any more united beneath the surface. The Group comprises nations as different as India, Brazil, Venezuela, The Gambia and Nepal, and so the diversity of need is enormous.

What they agreed upon

In the Programme of Action, delegates agreed, in essence, on the following:

- that the debate on the terms under which technology is transferred from the developed to developing world was not going to be resolved at UNCSTD; there are wide differences over questions of confidentiality, restrictive practices, the relevance of existing international agreements (at a time when the Group of 77 is promoting the New International Economic Order) and so on;
- that developing countries be exhorted amongst other things to:
 - formulate national science and technology policies;
 - make these policies as wide-ranging as possible;
 - establish policy-making bodies;
 - set up information networks, standardisation agencies, specialised institutions and so on;
 - formulate a technology transfer policy;
 - promote scientific associations, extension services, media reporting of science;
 - develop manpower policies and study the brain drain;
 - coordinate all this, and much besides, on a regional and international level;
- developed countries should help the developing countries along by supporting a new, additional funding facility under the aegis of the United Nations Development Programme. They should be invited to make pledges totalling \$250 million over the two years to 1981. This facility would finance a broad range of activities aimed at strengthening the endogenous scientific and technological capacities of developing countries. The Group of 77 had initially asked for \$2 billion per year by 1985;
- that a new UN inter-governmental committee oversee the implementation of the Programme of Action, and
- that a global information system be established.



In short, developing countries have been told to take science and technology much more seriously at a governmental level and to establish all sorts of new institutions. But the funding for it will very largely have to come from their own pockets. \$250 million is not a large sum when divided amongst all potential applicants. And furthermore there is a strong feeling both in developed countries and in the poorer and smaller developing countries that when it comes to sharing out the money, the larger and relatively richer countries of the Third World will dominate the handouts, because they are more easily able to put up an impressive case, already having the right beginnings.

Role of scientists

The Programme of Action was almost entirely debated at the diplomatic level. How then did scientists and technologists fare at the conference? There had been an ambiguity about their role in UNCSTD right from the word go. Naturally it was not to be just another scientific conference. But how deeply were scientists to be drawn into participation in Vienna? It has been no secret that Joao Frank da Costa, the Secretary-General of the conference has had little enthusiasm for their involvement, and certainly issues such as transfer of technology, and institutional and funding arrangements within the United Nations system were ones on which relatively few scientists might have an informed view. On the other hand, scientists might have extremely clear views on global information networks, means of strengthening facilities in the developing world, research priorities and so on. In the event, however, the ambiguity persisted right to the end of the conference, and left many scientists disillusioned.

One reason for disappointment could have been the variability in the delegations. Some were manned almost entirely by civil servants, others carried a professor or two and a very few (such as West Germany, Austria, France and the United States) made any serious effort to be multi-dimensional by incorporating politicians, academics, industrialists and trades-unionists. There was thus a feeling amongst scientists that they did not constitute a critical mass capable of making their presence felt. This sentiment undoubtedly coloured the discussions in the working group on science, technology, development and the future. Under certain circumstances it would have been possible for this working group's report to have provided the perfect technical counterbalance to the administrative and structural Programme of Action — in effect presenting a pair of papers outlining possibilities and how they might be achieved. In practice this was not to be.

Some of the responsibility for this must undoubtedly be placed on ACAST, the UN's Advisory Committee on Science and Technology. The committee hastily put together a colloquium of 300 scientists in Vienna for the week before UNCSTD. Creditable as such an occasion may have been (and there were a majority of developing-world scientists at it), it undoubtedly siphoned much

of the scientific interest away from UNCSTD. And since the working papers for it were prepared by the UN's special agencies, the colloquium already had the veneer of a UN activity before it started. Its final report merely confirmed this, being the sort of document that would be very acceptable in UN circles but which lacked a sense of novelty or character.

The UNCSTD working group started off by falling into the trap of wanting to submit a similarly dull document. But eventually a small group of scientists, clearly horrified at what they were in danger of having to put their name to, forced at least a modest revision (after the years of preparation and millions of dollars poured into UNCSTD, the group's activities were heavily curtailed by lack of time!). Ultimately the report did give a more satisfactory indication of how science and technology could contribute — with particular reference to the need to involve the young and to make positive efforts to encourage women. The role of the United States delegation was crucial — even during late-night negotiating sessions when almost every other delegation was either absent or fielding one representative, the US team would be up to ten in number.

Modest momentum

The cause of development is ultimately in the hands not of delegates to conferences but of politicians apportioning resources and of those at the cutting edge. Has enough come from UNCSTD to give them new direction, support and drive? The Chinese at UNCSTD produced a very apt proverb "don't add yet more flowers to the bouquet; send more charcoal in snowy weather". Will the conference be remembered just for verbal bouquets, or are developed countries prepared to send more tangible help? The level of financial support over the next two years leaves the question open, although the establishment of the funding facility itself must be regarded as a modest step forward in international collaboration. But much depends on just how effectively developing countries put together their proposals in the coming months. It would be easy to spend the money on large capital-intensive projects — grandiose new universities and research laboratories lavishly equipped with equipment but short on running expenses and staff, particularly at the technician level. By 1981 such demands could already have given a bloated and inappropriate image to the whole project. It is better that developing countries (and particularly the poorer developing countries) take the opportunity slowly and thoughtfully to lay down the foundations of a real science and technology policy. Much of the money would be better spent on encouraging communication and discussion than in bricks and mortar.

The outcome of UNCSTD has never been seriously in doubt — the Group of 77 have had to sacrifice their rosier dreams, developed countries have had to make some relatively minor concessions. What matters now is that the modest amount of momentum acquired is not squandered in the years ahead.

A slightly bolder approach

As long as *Nature* has existed, letters reporting important scientific advances have formed the backbone of the journal. The length of these communications has grown steadily with the years as scientists have felt under greater pressure to give sufficient detail to allow a critical evaluation, but their style has remained remarkably unchanged.

We are often asked why we do not fall into line with almost every other journal and publish an abstract with each letter — and our answer has been that we have tried to preserve in the letters section the sort of directness that people achieve when writing to each other. But we go on to add that there is nothing to prevent anyone writing a first paragraph that encapsulates the whole story — background, summary of the work done, consequences;

something more valuable than an abstract because it gives context.

For the past few years we have gradually been trying to persuade authors to improve their first paragraphs, if necessary with some help from our sub-editors. Now we take the experiment a bit further. From this week's issue, the first paragraph of each letter will be printed in a bolder face. We hope this may provide some little help to those very many of our readers who turn to *Nature* to keep in touch with a wider range of science than their own specialisation. We hope too that our authors will be encouraged to put even more thought into getting the first paragraph right. And the writer of this leader might practise what he preaches. □

UNCSTD agrees on new committee and fund

Two major decisions were agreed at the final plenary session of the United Nations Conference on Science and Technology for Development which ended in the early hours of last Saturday morning. The first was to set up a new intergovernmental committee on science and technology for development, open to all member states of the UN. The second was to recommend the establishment of a new financial system for science and technology for development and, while the details of this are being worked out, to set up an interim fund based on voluntary contributions, with an initial target of at least \$250 million for the two year period 1980-81. Both these decisions were modifications of the Group of 77's original demands.

The Group of 77, for example, had originally proposed that the intergovernmental committee be set up by, and report back to, the General Assembly of the UN. But the US had insisted that the committee be established by the UN's Economic and Social Council (ECOSOC).

A compromise was suggested by the Common Market countries and eventually accepted by the conference, along lines drawn up by the British delegation. The new committee will be set up by the General Assembly, but it will report back to the General Assembly through ECOSOC. And the latter, while not permitted to alter the reports, will be able to comment.

The new committee will play an important role within the UN system. How effective it will be will depend on factors such as the strength and status of the support it is given from the UN, which is yet to be decided. One of its main purposes, according to the wording of the final conference decision, will be to "help the General Assembly formulate guidelines for harmonising the policies of the United Nations system in regard to scientific and technological activities".

Specific responsibilities will include: identifying priorities for activities within the programme of action agreed by the conference (now referred to as the Vienna programme of action), preparing a plan for carrying out the programme, and monitoring activities and programmes related to science and technology within the UN system.

A particularly important function of the committee will be to "give directives and policy-making guidance" to the new financing system which the conference proposed that the UN should establish (an earlier proposal from the Group of 77 had been that the committee should "direct"



the way that the financing system was operated, but this language was rejected as too strong).

The financing system agreed is considerably less than the original demand of the Group of 77. This was for a target of two billion dollars by 1985 and four billion dollars by 1990 reached through "automatic" contributions mainly from developed countries.

The main opposition from the developed countries to this proposal was based more on the manner in which contributions were to be made than on the size of the fund. Almost all objected to the idea of "automatic" contributions based on factors such as the balance of trade in manufactured goods — a type of international tax which was strongly resisted — and it was agreed that at least initially all contributions will be voluntary. Other countries, in particular various members of the EEC, were initially opposed to the idea of a fund at all but were eventually convinced to accept the general consensus.

The purpose of the proposed global financial system, according to the conference resolution, will be to "finance a broad range of activities aimed at strengthening the endogenous scientific and technological capacities of developing countries and, in particular, assist in implementing the programme of action".

It was agreed by all conference participants that factors to be taken into account in determining the nature and level of the system's resources should include: the asymmetry of technological capacity between developed and developing countries, the need for predictability and continuous flow of financial resources, the need for substantial resources additional to those existing within the UN system and finally the need for untied external resources.

Since it will take some time to make the arrangements for this financing system, an interim fund, to be administered by the United Nations Development Programme, will be created: and a target for voluntary contributions to this fund has been set at "no less than \$250 million" over the two years 1980 and 1981.

A conference will be held before the end

of the year at which countries will indicate how much they are prepared to contribute to the interim fund. The main potential donors are the US and Saudi Arabia, with promises from France and a number of smaller European countries. Precisely how the interim fund will be spent remains to be worked out. But it is thought that most of it will go towards helping to build up scientific infrastructures in developing countries.

David Dickson

What scientists did in Vienna

WHILST there was no doubt that the main focus of attention at UNCSTD would be the debate over the Programme of Action, with its associated battles over institutions, money and so on, it was planned that there would be a parallel session to use the assembled scientific and technological expertise to good effect. This session had two fields to look at: new science and technology for overcoming obstacles to development; and science and technology for the future. The working group was chaired by M. Malitza of Romania.

The group had at its disposal national papers, together with two specially commissioned studies and the reports of several conferences. It also had freshly to hand a report from ACAST, the UN's Advisory Committee on the Application of Science and Technology which had met in Vienna the previous week.

The working group (which comprised as many delegates as cared to show up) always seemed to be loaded with disadvantages. It did not start meeting till the fourth day of the conference, and then in a building not within walking distance of the main conference centre; it was presented with a bland ACAST report which seemed to convince the working group that its report should be equally platitudinous; and whilst a few delegations (France, Germany, Austria, United States in particular) had a good complement of scientists and technologists, many could field at most one, more often none at all.

The first draft of the report reflected

these weaknesses. The United States, which had not been one of the 6 members of the drafting group and which claimed it was having difficulty in getting its views considered, was particularly severe in its strictures that the report lacked 'soul'. As one of its delegates put it, "we have put a big effort into this conference; how can we defend this document to our scientists and technologists back home?" The British, too, were voicing concern both publicly and privately that "with all the expertise here in Vienna" nothing more helpful was emerging for consideration in planning future development programmes.

Certainly much of the document merely repeated the same general principles as were being negotiated more formally in the Programme of Action elsewhere. But by this stage there was not enough time left to start again from scratch. Nor would many developing country representatives have been pleased at the prospect, as they were more in need of a general statement of principles to put to the government back home rather than a 'soulful' document.

In the event, and amidst much lobbying by the US, a second draft was produced which left the Americans moderately satisfied without causing problems amongst developing countries. Some of the key points are outlined below.

Science: 'Science policy should be mainly linked to long term objectives'. 'Science and technology . . . should be consistent with national aspirations and objectives'. 'Science of the future must include in its concerns the uplifting of the welfare of the total human being' (the first version had contained the phrase 'basic science should be left free to follow its own internal dynamics').

Obstacles: 'One of the most urgent questions is disarmament . . . more effect should be given to analysing in what ways ongoing research and technical application now directed towards war could be adjusted for peaceful applications'. 'The brain drain of the most skilful scientists and technologists from developing countries creates a serious obstacle to their own scientific and technological development . . . developed countries should assist by actively discouraging a brain drain from developing countries'. This last phrase was concocted as an alternative to the more loaded phrase 'should assist by not enticing scientists and technologists from developing countries', to which some developed countries took exception. But at least one delegate was wondering privately whether 'discouraging' the brain drain did not contravene agreements on the free-flow of scientists.

The United States would additionally have liked a reference to the population problem, but could not get a consensus.

Objectives: 'All countries should develop

G77 wins on global information network

ONE of the few victories of UNCSTD, from the point of view of the developing countries, is the agreement in principle to set up a global information system for the benefit of the Third World. This was a hard-fought victory, with resistance from the US, Canada, Japan and the EEC. And it is a victory that also places responsibility on the developing countries to evolve extensive national systems.

The developed countries had argued that systems which operate with such low efficiency in the developed world, with all possible sophisticated backup, have little chance of operating efficiently in the third world. But after what amounted to the Group of 77's toughest stand at UNCSTD, the developed countries agreed to develop a global information system within the United Nations system, having a global focal-point and series of networks including national focal points.

The function of the global focal-point caused much controversy. The developing countries wanted to perform both retrieval and referral functions. The developed countries believed it was not technically possible for the global-point to perform a retrieval function.

After four days of negotiation in closed committee meetings, the Group of 77 finally dropped its demand. It was decided that the focal point will perform the referral function, but will also act as a

"complaints bureau". The retrieval function will be performed largely by the national focal points, but users facing difficulties in getting a response would be able to refer to the global focal-point.

For the global information network to function properly, the national focal-points must provide significant backup support. The Third World countries are required to extend and develop their national information systems considerably, and to have storage and retrieval facilities on information concerning development of science and technology, foreign sources of technology supply, sources of foreign capital and their conditions, and national users of science and technology. In their turn, the developed countries would extend their own facilities for use by developing countries. They also agreed to provide, "the fullest possible access to available information technologies".

Furthermore, the developed countries would allow access to research and development information relevant to the social and economic development of developing countries". Who decides what is relevant and what are the criteria of relevancy, however, were not significantly discussed. Also omitted from the general agreement is the question of confidentiality.

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endogenous capabilities to assess the trends of science and the consequences of technological options'. 'Developing countries have to carry out their own studies of global problems so as to bring their own perspective to bear on the world *problematique*'. 'Goals include overcoming poverty and seeking ways to reduce the disparities that exist between developed and developing societies in, for example, life expectancy, infant mortality and education'. A stronger version speaking of 'halving by the year 2000 the disparities' could not attract a consensus.

Resources: 'As concerns R and D activities in developing countries, every effort should be made by the world community to raise the level of these activities from the present very low level to a substantially increased level. It was the considered view of many delegations that a more quantitative goal should be set and they felt that this should be of the order of 20% to 25% of global world research and development expenditure by the year 2000'. Countries such as India and Bangladesh were keen to have a target, but there were doubts amongst some countries about the reasonableness of these targets or even the precise definition of the terms used.

Action: The building up of endogenous capabilities requires 'massive expansion of

the educational system in order to provide access to primary, secondary, technical and higher education to most, if not all, the populations, male and female equally'.

Governments in developing countries should provide support both for existing and new centres of excellence 'dedicated to both basic research and applied research of relevance to their societies'.

Scientists in developed countries 'should be encouraged and helped by their governments to take a more active interest in solving problems in developing countries . . . exchange of scientists and technologists between developed and developing countries should be encouraged . . . informal communications between scientists based on mutual interest are also among the powerful instruments which can be used to help in problem solving'.

Specifics: The working group made sure, in a footnote, that just about every branch of science and technology with the remotest relevance to development received a mention. But it also identified in the main text some rapidly developing fields such as information technology, non-conventional generation of energy (including fusion), biotechnologies and marine sciences.

Women and young scientists: 'Technological development often affects men and women differently and the intro-

duction of new technologies has tended to have an adverse impact on the latter, thereby lessening their earnings and social status. It is therefore of the utmost interest to society that in future the full participation of women be ensured in the planning and setting of priorities for research and development as well as in activities relating to the design, choice and application of science and technology for development. They should also be provided with equal access to scientific and technological training and professional career opportunities'.

'Rapid development of science and technology throughout the world will

depend in part on the younger men and women who can be brought into these fields, involved in decision making bodies and given full opportunity to use their intelligence and skills'.

Institutions: 'There is a need to restructure and strengthen the international scientific and technological system and the role of international intergovernmental organisations in such a way as to permit such organisations to act as effective instruments in satisfying the priority objectives of member states'.

'An appropriate UN body on science and technology, and other UN agencies competent in this matter' should make

recommendations regarding changes in the structure of science and technology to 'service the needs and problems of the peoples of the world, with special regard to the needs of developing countries'. Originally this recommendation spoke only of the new intergovernmental committee that was being established elsewhere in the conference, but the Soviet delegate, in a rare intervention, asked for the involvement of other UN agencies. This possibly keeps the door open for UNESCO and ACAST, both of which had been widely thought to have lost considerable influence as a result of this conference.

David Davies

EEC countries attempt to speak with one voice

ONE of the most significant consequences of the UNCSTD conference is that, for almost the first time, the nine member countries of the European Economic Community were required to speak with a single voice on the appropriate international policies for applying science and technology to development.

At times, EEC manoeuvring to achieve this unity resembled those of a cox-less, novice rowing-eight (Luxembourg played a relatively minor role in the negotiations), with everyone heading roughly in the same direction, but with substantially different ideas about commitment and timing. Furthermore, the political requirement for solidarity during formal negotiations with the developing countries meant that many harsh words were said to colleagues behind closed committee doors.

The outcome, however, was at least a partial victory for political cohesion. And this will inevitably have important consequences for the future role played by EEC countries in encouraging particular forms of economic and political development in the Third World.

The pressure to speak with a common voice added a third political factor to the two which have traditionally dominated national discussions of foreign aid policy: on the one hand, the domestic implications, both political and economic, of spending public money on foreign aid, on the other, the international gains to be made by spending this money in a particular way.

A fourth factor played a less explicit, but no less significant, role during the conference negotiations. This was the knowledge that the US had committed itself to ensuring the approval of an interim \$250 million fund, with particular executive responsibilities given to the United Nations Development Programme, and investigating the setting up of a long-term international funding system for science and technology for development.

Caught between these four different sources of political pressure, each member country of the EEC was forced to weigh up their relative importance in its own

particular circumstances — and decide its negotiating strategy accordingly. At the one extreme Denmark had, at the beginning of the conference, surprised some of its EEC colleagues by advocating immediate commitment to the US-backed package. Compromise was "imperative" to make UNCSTD successful, the Danish Minister for Foreign Affairs, Mrs Lise Ostergaard, told the plenary session.

Similar indications of support came from the Netherlands and Italy. Holland was eager for domestic political reasons not to appear to be backing away from its reputation for generosity in foreign aid, despite economic difficulties.

In contrast, the three major economic partners in the EEC — Britain, France and Germany — each had different reservations about the US-backed package. Britain's position was made clear by the Minister for Overseas Development, Mr Neil Marten, who told the conference that recent drastic cuts in public spending meant that Britain had no money to contribute to any new fund, and that in any case Britain had "serious doubts" about the value of any new financial mechanism or the separation of funds for science and technology for development.

At the same time, however, the British delegation was keen to maintain the political unity of the EEC's negotiating positions and not to be seen as the only country which was sticking out against a consensus.

Germany's position was more complicated. Politically it agreed with the US's view about the need for "cooperation" rather than "confrontation" in economic relations with the developing countries, and about how science and technology agreements can help achieve this goal. It also accepted the need for EEC unity, and could not complain about lack of funds.

For domestic political reasons, however, the German government was reluctant to provide funds to a programme whose precise activities had not been spelt out in detail. It tried — unsuccessfully — to get the EEC to back the idea of contributing

directly to a World Bank research programme on renewable energy resources for developing countries.

Finally France, the maverick in the group, was happy to be seen increasing its foreign aid contributions, but less happy about a sectoral fund inside the UNDP. The French annoyed some other EEC delegation members by announcing to them two days before the end of the conference its opposition to the new fund.

Despite such divergences of opinion, by the end of the conference a consensus had been reached to support the US/UNDP package in principle (see page 2). At the closing session, however, it was publicly acknowledged that there remained differences between the EEC members. Speaking on behalf of the whole group as current president of the EEC, the head of the Irish delegation said that six countries — Belgium, West Germany, France, Ireland, Luxembourg and Britain — wished to make it clear that they maintained "their well-known reservation on the efficacy of any sectoral fund for science and technology for development". However, in the interests of achieving a consensus, the statement said, these six countries had not opposed the arrangements for an interim fund, once it had been established that the contributions would be on a voluntary basis.

No-one within the EEC delegation was entirely happy with the outcome. But it was generally accepted that, given the pressures under which the group was working the outcome as a political exercise was fair.

Whether the result will be of overall benefit to the developing countries is another question. Some EEC delegates point out that by agreeing to accept a consensus, some countries adopted a more conciliatory position than they might otherwise have done. Developing country critics, however, also argued that through such consensus-building, countries which might otherwise wish to adopt a more radical stance in their general approach to north-south relationships might be persuaded against doing so.

David Dickson

Very high radiation levels found in Swedish houses

FOLLOWING the discovery of extremely high concentrations of radioactive radon daughter elements in Swedish houses, a government committee has proposed measures to allow maximum concentrations far higher than would be internationally acceptable. The radon producing the daughters derives not only from radium in the soil and rock on which the houses are built, but also from radium in building materials.

The immediate cause of the high levels recently recorded, however, is energy-saving. Enthusiasm for energy conservation has led many people to tape over their windows to keep the warmth inside; consequently ventilation rates have dived. With a ventilation rate of one air exchange every two hours, even the worst radon daughter levels are greatly reduced; but some of the houses measured have had only 0.2 air exchanges every two hours.

To overcome the conflict between energy saving and health, the committee suggests the installation of air exchangers in which the outgoing air would warm up the incoming air. Discussions are currently being held to decide how much financial assistance could be paid to house-holders for the necessary alterations, but at the moment it looks as though the amount will be small.

From the radon point of view, the worst building material used in Sweden is aerated concrete made from alum shale. Its manufacture was banned in 1974 for this reason, but it had been a popular building material

for 45 years. At the other extreme is wood which contains practically no radium. Ironically enough, the houses whose high radioactivity led to the committee of enquiry were built of wood — but constructed on slag heaps left after the mining of alum shale. Only a small proportion of risk houses have so far been identified, and campaigns are being organised to find others. Current estimates of the numbers of houses that will have to be altered run as high as 15,000.

The International Committee on Radiation Protection recommends that no member of the general public should be exposed for many years to an annual dose equivalent of more than 1 millisievert (mSv) of radiation, excluding natural background and medical sources. This level is estimated to give a lung cancer rate of one person in every thousand exposed for 60 years. There is as yet no internationally-agreed level for exposure to radon in houses. In the average Swedish house, the concentration of radon daughters gives a dose equivalent of 3 mSv/yr, assuming a person spends 80% of time indoors — and the worst cases found in the present investigation are as high as 100 mSv/yr. "If nothing is done, our present building situation may cause up to 1100 cases of cancer in about 30 years", says the Director of the Radiation Protection Institute, Professor Bo Lindell.

The committee proposes that nobody in existing buildings should, over the next five years, be exposed to a radon daughter level

of more than 2000 bequerel (Bq) year/m³, a total dose equivalent of 285 mSv over 5 years. Existing houses should be altered before their inhabitants have received this total dose. This exposure will, it is estimated, mean that three people in every hundred will develop lung cancer after 60 years. As far as new buildings go, the committee proposes radon daughter concentrations of 70 Bq/m³ (9 mSv/yr) and at this exposure level, one person in every hundred will develop lung cancer after 60 years. According to the committee, these dose rates are the best that can be achieved at the moment, given the level of radioactivity in Swedish rock, soil and building materials.

The number of people who will develop cancer because of the high radon daughter levels may however be less than these estimates. According to a spokesman at the Radiation Protection Institute, current research into the risks of contracting cancer at certain concentrations of radon daughters shows that the risks to the ordinary man may have been overestimated. The risk estimates are based on studies of uranium miners, the concentrations around miners who developed cancer being extrapolated to the ordinary population. But these studies did not take into account the fact there is always more particulate matter in the air in mines than in ordinary houses. As radon daughters enter the body attached to airborne dust, the risk to the general public may have been overestimated.

Wendy Barnaby

Women in science: some improvement, but still a long way to go

There has been a considerable improvement in the status of women PhDs in academic science over the past decade, but further substantial gains are still needed before equal opportunity is fully realised, according to a report published recently by a committee of the US National Academy of Sciences.

On the positive side, the report, prepared by the academy's committee on the education and employment of women in science and engineering, says that in the past decade women's share of all science doctorates has doubled, from 9% to 18%, and is still increasing in all fields of science.

Furthermore women are occupying a growing proportion of research faculty positions, particularly in the larger institutions; in the top 25 research universities, for example, women account for 35% of the growth of science faculties since 1973.

However there remain several areas of concern. One is that there continues to be a disproportionate number of women in two kinds of position: part-time instructors or lecturers which are outside the tenure stream offer little chance for productive

research; and postdoctoral or research staff positions.

There are also areas in which the discrepancy between salaries for male and female research workers is giving continued cause for concern. The report points out, for example, that although the difference in postdoctoral stipends decreased from 5.7% to 4.9% between 1973 and 1975, it had increased again to 7.5% by 1977.

"On the basis of available data, it appears likely that at least a large part of the salary differences between men and women postdoctorals derives from bias. At this level no significant differences in overall ability or promise can be documented, and male and female scientists should be rewarded equally for comparable work. Systematic salary differences at this early career stage are important not only for their immediate relevance to equity, but also as a portent of future status," says the report.

Among the major conclusions of the report are that:

- rank for rank, women faculty continued to be tenured less often than men. For all ranks, 72% of the men but only 46% of the

women hold tenure appointments, and this disparity is increasing

- in relation to the pools of new PhDs in the various fields, chemistry and mathematics employ far lower proportions of women faculty than other fields

- women account for all of the net growth in science faculty at the assistant professor rank for the top 50 US universities (by R & D expenditures) and for nearly half of the increase in all other institutions

- wide field of variations in rank, salary, and tenure distribution for women faculty compared to men suggest that an assumed lack of mobility of married women is at most contributory rather than a primary reason for women's evident disadvantage.

The continued plight of women in science was emphasised at recent hearings before Senator Edward Kennedy's health subcommittee by Ms Anne Briscoe of the Association for Women in Science, who claimed that despite the results of affirmative action programmes, sexist attitudes were still deep-rooted. The health subcommittee is currently discussing a "Women in Science and Technology Equal Opportunity Act". □

news in brief

Silicon production costs 'reduced 90%': A new process, developed by a group of researchers at the Stanford Research Institute, California, may reduce the cost of silicon by as much as 90%, an *SR1* publication has claimed (*The Leading Edge*, summer 1979, p22). Thus, by 1986, 1 kg of silicon could cost as little as \$5, says the article. At the moment, 1kg of silicon costs \$60. Costs could be further offset by the sale of sodium flouride, a by-product of the new process, for other industrial uses. The new manufacturing method is a one-step process producing relatively pure silicon, with sodium flouride as a by-product. The necessary chemical reactions take place in one vessel and the cheapness and ease of the new process are further enhanced by the fact that the reaction is exothermic and therefore requires no outside fuel source. The main beneficiary of a new breakthrough in the manufacture of silicon would be solar energy using cheap silicon cells. However, the new process is yet to be tested on an industrial scale.

Development need not endanger the environment:

Environmentally 'safe' development can be carried out by all nations according to Dr Mostafa Tolba, (right) Executive Director of the United Nations Environment Programme (UNEP). In an interview with *Siren*, UNEP's Regional Seas Programme newspaper, Dr Tolba said



"appropriate environmental management should be a common practice to both poor and rich nations, and should be the meeting ground of common objectives." Developing countries should seek a solution to their environmental problems in the process of development itself. "It is not so much a question of financial resources necessary to meet environmental problems, as it is the undertaking of rational decisions based on a long-term integrated concept of development."

Dr Tolba felt that the regional approach to environmental problems was one of the most successful. UNEP's Regional Seas Programme is "one of the best of our programmes if not just the best". This programme aims to protect the enclosed seas and coastal environment where some of the highest levels of pollution are found. Compatible research will be carried out throughout these regional seas enabling a communal solution to the problem to be made. This, together with a common resolve among coastal states towards problems affecting them all, are the two most important practical achievements of UNEP's Oceans Programme according to Dr Tolba.

Aldermaston deaths: Two former workers at the UK Atomic Weapons Research Establishment, Aldermaston died from cancer last month with more than the permitted level of plutonium in their lungs. Internal organs from both men's bodies have been sent to Windscale for tests, but it is not yet certain whether radiation caused their deaths. Kenneth Cummins, 49, had been at AWRE for 14 years and Albert Newman, 51, had worked there for 26 years. Both worked in the "active areas" of the establishment and were among the 70 men claiming damages against the Ministry of Defence for plutonium contamination (19 July, p184). Parts of Aldermaston were closed last year after 12 workers were found to have more than the permitted level of radiation in their bodies. Before these areas can be re-opened, new ventilation methods must be installed and more safety and maintenance workers must be employed in accordance with the findings of the Pochin report which examined radiological health and safety at the establishment and was published at the end of last year.

Finnish reactor delayed over safety requirements: Delays in commissioning the second set of the Loviisa nuclear power station are, it appears, due to the higher safety requirements demanded by Finland. The equipment for Loviisa, produced in the Soviet Union, includes a number of modifications not considered necessary for Soviet home consumption. These include a containment vessel and a shield to prevent internal corrosion of the reactor's pressure chamber.

In mid-June, the Finnish power authority Imatran Voima announced that the loading of the second set had been postponed, "since talks between Finnish and Soviet specialists on the removal of the shield had not yet been concluded." It was not clear at that stage why an anti-corrosion shield should need to be removed at all, although the Finnish media stressed that Imatran Voima "wished to observe strict safety standards" and "in case the chamber needs to be repaired" were ready to postpone the loading till August "or ever later".

The most recent news from Imatran Voima reveals that the authority had applied to charge the set in June, but the Radiation Protection Institute had delayed permission, after cracks had been found in the "inner anti-corrosion coating of the vessel" (presumably the "shield" referred to in earlier statements). These cracks, it was alleged, were due to slag "inclusions". The Soviet suppliers have offered an explanation of these faults.

Air pollution complaints increase: The number of complaints about air pollution made against industrial works in the UK increased in 1977 despite an overall reduction in air pollution, according to a Health and Safety Executive report published recently. Mineral works were the worst offenders accounting for 97 of the complaints. The Chief Inspector of HM Alkali and Clean Air Inspectorate, Frank Ireland, said "We receive far more complaints today about far less pollution compared with conditions even 10 years ago when the situation was much worse." More inspections, stricter standards and greater public awareness probably accounted for this increase.

There was also a slower rate of implementation than expected of new emission standards set by the Alkali and Clean Air Inspectorate in 1977 due to the economic depression. Some old plants were kept active to keep staff in employment, particularly in the iron and steel industries, and other industries were allowed longer to implement the new standards so that they could spend scarce resources on creating or maintaining existing jobs. Existing standards, however, were never relaxed and in many cases emissions were considerably better than requirements. *Health and Safety: Industrial Air Pollution 1977*, HMSO (London) £3.

Pioneer 11 discovers new ring of Saturn: The Pioneer 11 spacecraft, which successfully flew past Saturn last Saturday, has discovered a fifth ring, the "F" ring, lying outside those already known and circling the planet. Attempts to find evidence of a broader, more diffuse "E" ring have given only ambiguous results so far. The gap between the "A" and "F" rings has now been labelled the Pioneer Gap. Photographs returned from the spacecraft also indicate the presence of a dark blue jet stream with greenish tinges near Saturn's north polar region and of a possible new satellite. The satellite which appears faintly in photographs has yet to be positively identified: it may be a newly discovered moon or possibly some known satellite appearing unexpectedly.

Short wave radio fools Fire Brigade: A short wave radio in the Israeli Embassy in London is now thought to be the cause of the radiation detected recently at Kensington Fire Station (30 August, page 715). The National Radiological Protection Board called in by the London Fire Brigade found no unusual levels of radiation and thought interference from radio waves may have caused the fire brigade equipment to register radioactivity.



Students meet before the conference: social values are not being taught with technology



Scientists and clergy debate global problems

Scientists and theologians from the First and Third Worlds met recently to discuss the social impact of western science and technology. Much of the debate was heated as **Dr A R Peacocke**, of Clare College, Cambridge University, reports.

AN unprecedented world conference on 'Faith, science and the future', organised by the World Council of Churches, was held at the Massachusetts Institute of Technology last July. It drew many threads of the WCC's previous work together and was structured around four central themes: science and faith, questions of ethics, of political economy, and plans for Christian action in relation to science and technology.

Three hundred and thirteen delegates from 56 countries attended. About half of them were scientists and technologists and half were theologians, pastors, social philosophers and politicians. More than 100 students also attended. Many church affiliations were represented and a few representatives from other religions, including Islam and Buddhism, were present. With such a mix, it is not surprised that strong feelings were expressed, especially when topics for discussion included nuclear weapons, genetic engineering, nuclear and other forms of energy and the impact of western science and technology.

Delegates from the 'Third World' were about a third of the total and rapidly constituted themselves as a pressure group. It soon became clear that they experienced 'science' primarily as technology, a technology moreover that is having an enormous and disruptive effect on their societies, with the transnational corporations cast as the principle agents of their ills. Their frustration and sense of exploitation was expressed in a resolution (not a resolution of the whole conference) in which a number of them said "we denounce the historical and current use of science and technology by industrially and technically advanced societies, to serve military and economic interests which have brought about great sufferings to the people of the Third World. This has been done in the guise of an ideology of

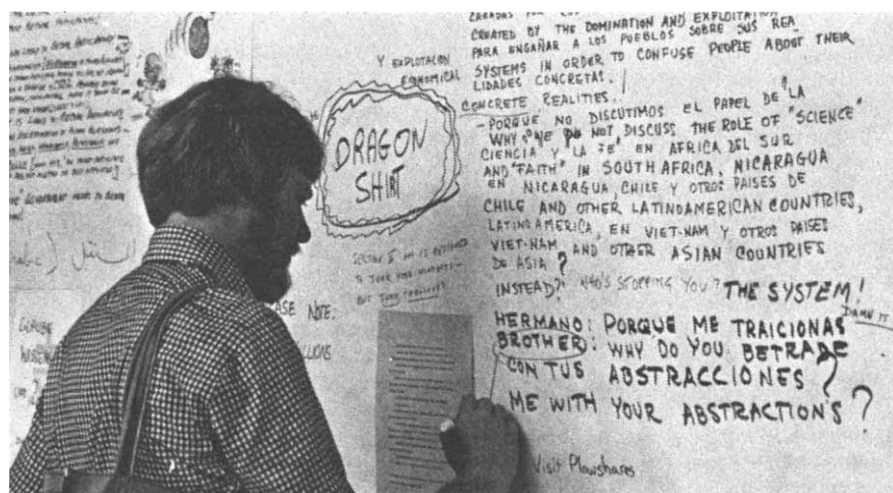
objectivity and value-free pursuit of truth'. These attitudes produced first shock and then strong opposition from western (or, more accurately, northern) scientists and others present who regarded the pursuit of knowledge of the natural world as valuable in itself and knowledge, once achieved, as ethically neutral.

This particular tension became apparent early in the conference in the contrast between the account of science, given by Professor R Hanbury Brown (astronomer from Sydney University), as "a social activity in which we seek to discover and understand the natural world" and that of Dr Rubem Alves (philosopher, Brazil) for whom "most of the explanations that science proposes about itself are not only untrue; they are dangerous", as dangerous as a wolf to Third World lambs. There was clearly a wide gulf of misunderstanding between these two typical western and Third World perspectives on science — and indeed technology should have been substituted for science in many of the discussions and even in the conference title.

It is hard to judge if this gulf was really

bridged, but the western-trained scientists, technologists, economists and theologians were uneasy with the inadequate treatment, especially in the section meetings, of conceptual and critical issues such as the status of scientific models, the limitations of scientific knowledge and how scientific research projects are engendered and financed. However this was inevitable in such a mixed gathering and the concern was expressed in section recommendations which requested the WCC to initiate smaller and more professional study conferences on particular assigned problems. These kinds of recommendation are more likely to be implemented than worthy, but doomed-to-be-ineffectual, instructions to those outside the churches such as the recommendations of one section to governments on nuclear energy, and those of another to "governments, industrial companies, universities and scientists" to make scientific and technological information more accessible to all citizens.

Matching in intensity the Third World issue, was the desire of the delegates to



The conference 'wallnewspaper'

show their unambiguous support for nuclear disarmament. Confessing "the part played by science in the development of weapons of mass destruction and the failure of the churches to oppose it", the delegates were unanimous in their endorsement of a resolution (drafted by a committee chaired by Dr J Habgood, Bishop of Durham) that called for full implementation of SALT II, advocated SALT III, supported UN initiatives on disarmament and opposed the development of new nuclear weapons. However, one proposal for unilateral disarmament was overwhelmingly defeated.

The other aspect of the nuclear problem, the 'peaceful' uses of atomic energy, also led to lively controversy. Professor David Rose, of MIT, argued for the continued use of nuclear energy at least until solar energy is developed, which means in practice, for several decades. But Professor J Rossel, a Swiss physicist, argued equally strongly against its use. Not surprisingly the section report concerned with this issue was divided and at the plenary session a keen debate resulted in the passing of a resolution, by a 129-45 vote, that called for a 5-year moratorium on the construction of nuclear power plants. My impression was that this majority included a large number of votes from among the 100 student representatives who, although neither strictly delegates of their churches, nor appointed as experts, had full voting rights.

The students, from 55 nations, had met before the conference at Wellesley College, Boston, to discuss the key issues. They asserted *inter alia* that science education is not tying social values to the teaching of technology and this theme was also heard when they reported their views to the main meeting. They were again especially critical of science education which they saw as an elitist weapon used to preserve power and institutionalised values.

This raises the wider question of how representative the conference was. But, representative of whom? Certainly it voiced the profound concerns about science and technology of many active members of the Christian churches across the world, which, it must be stressed, are increasing rapidly in the Third World. It is difficult for such a conference to claim to be 'representative', but it might nevertheless aspire to leadership of opinion resulting in action. If it does, the hopes over many years of the devoted staff of the WCC (and one thinks particularly of Dr P Abrecht, the director of the Church and Society unit that was responsible) will have been justified. And, in the end, the most remarkable thing about this unique conference is not so much its plethora of resolutions and its section reports (yet to be published) but the fact that it was held at all — and in MIT. The relation of the Christian churches to modern science and technology can never be quite the same again. □

Can biogas provide energy for India's rural poor —

or will it benefit only richest 10-12% of rural families? **Anil Agarwal** reports on the Janata government's plans to promote the use of biogas plants

INDIA has the world's second largest biogas programme, surpassed only by China, and its interest in bio-gas has probably the longest history. Although the first bio-gas plants were designed in 1939, the Khadi and Village Industries Commission was able to establish only 6,000 plants by 1974. The energy crisis of that year, which sent the price of kerosene soaring above the purchasing power of rural Indians and pushed them further into the environmentally disastrous use of firewood, persuaded the government of India to promote bio-gas plants more seriously.

In the last five years, over 75,000 bio-gas plants have been set up, and during the sixth five year plan (1978-83), the government hopes to establish 500,000 plants at a total cost of Rs2175 million (Rs17.7 = £1). About a third of this sum will be provided by the KVIC and the Ministry of Agriculture as grants, the rest being raised through loans from banks. These plants will create an annual production capacity equivalent to 750 million litres of kerosene. In addition, they will generate about 12 million tonnes of organic manure, which otherwise would have been burnt as cowdung cakes for fuel.

The programme should provide jobs for over 2,000 educated unemployed people and some 40,000 skilled and semi-skilled workers.

But compared to the total bio-gas potential within the country, the current rate of utilisation is extremely slow, much slower than China, which is reported to have set up over seven million bio-gas plants in a few years. Mr T.L. Sankar, secretary of Planning Commission's working group on energy policy, estimates that India has a potential of at least 18,750,000 family size bio-gas plants and 560,000 community size plants (with daily capacities of 1.7 and 142 cubic metres of gas respectively). While the family size plants could be used by richer rural households (with 4-5 head of cattle), the community size plant could supply energy to poorer households.

If this potential were realised by 1990, bio-gas could supply India with an energy equivalent to nearly 44% of its projected electricity consumption, and reduce its

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Mound of cowdung cakes: a traditional source of fuel

projected consumption of coal by 15% and firewood by 79%. The use of firewood would be reduced to an environmentally safe level, saving forestry expenditure, and the organic manure by-product would also reduce expenditure on chemical fertilisers. Although the investment required to adopt this option would be very high, about Rs 66,000 million. Mr Sankar believes that an energy policy that places high emphasis on bio-gas plants would have the "highest cost-benefit ratio".

To promote the use of bio-gas plants, the KVIC has started a self-employment scheme under which educated unemployed persons are being given training in installation and commissioning. They are paid Rs180 for supervising the installation of each gas plant. The KVIC takes a deposit of Rs50 as security before they begin work and retains 25% of their supervision charges until the total security reaches Rs500. These 'motivators' are expected to rectify all defects in the plants they have supervised, within the first three months of commissioning, at their own cost. Through this scheme, the KVIC hopes that greater care will be taken at the time of construction and thus maintenance and breakdown problems should be reduced; it claims that this scheme has given "very good results". Over 1500 motivators have already been trained.

Problems of managing community plants

But achieving Mr Sankar's target could be very difficult. There appear to be serious socio-economic obstacles to the widespread use of bio-gas plants. Past experience has shown that small family-size plants can be used only by the richest 10-12% of rural families. The large-scale use of bio-gas plants could drive poorer families to a greater reliance on the dwindling

supplies of firewood, as their present cowdung fuel becomes unobtainable.

Ironically, while the Chinese have unhesitatingly promoted the use of small plants, appropriate technologists in India have argued that only large community plants can benefit the Indian rural poor. But such plants require great managerial talents for their maintenance and operation, for the organisation of the collection of cowdung, valuation of the dung which would have to be purchased, and sale of the gas and manure generated. Furthermore, sociologists still have to explain what motivated those farmers who have bought bio-gas plants. While some reports say that the initiative comes largely from women, who like bio-gas as a clean fuel, other reports claim that farmers buy bio-gas plants because it provides them with organic manure for their fields, and regard the gas only as a by-product.

With financial assistance from UNICEF, two large prototype plants (30m³ and 45m³ respectively) have been built in the village of Fatehsingh ka Purwa in Etawah district. Bio-gas from these plants will provide cooking energy for the 200 inhabitants of the village as well as energy for running threshers, chaff cutters, mini-four mills, and for pumping water and generating electricity for street and home lighting. Fatehsingh ka Purwa is mainly a village of cowherds and the ratio of cattle to man is relatively high, which makes it possible for bio-gas alone to supply energy for so many activities. The total cost of the project is Rs158,000. Each family will receive slurry from the bio-gas plants in proportion to the cowdung it supplies. Bio-gas will be supplied free for two years and thereafter the villagers are expected to contribute. Few villagers at the moment appear to be prepared to pay anything for their energy, the concept of paying for fuel being traditionally alien to them. But they should eventually pay Rs10-15 every month per family. This is only one-tenth the depreciation and interest on the project, which thus involves a considerable subsidy. The Planning, Research and Action Division (PRAD) of the State of Uttar Pradesh, which is organising the project, has hired a manager for the plants and a trust of villagers has been formed to oversee it.

Meanwhile, the Department of Science and Technology is supporting an extensive R&D programme to develop cheaper designs of bio-gas plants and identify new feedstocks which can replace or supplement cowdung. Two important designs have been developed: one by PRAD which is an improved version of a Chinese design supplied to Pakistan, and the other an indigenous design developed by the KVIC.

In the standard KVIC design, the fermentation pit (also called the digester) and the gas holder are two separate structures, the mild steel gas holder floating over the fermentation pit. This design is efficient, gas-tight and provides gas at a

Earthscan

A conventional KVIC bio-gas plant with a mild steel gas holder

constant pressure, but the gas holder is susceptible to corrosion and cannot be made in the village.

In the Chinese design the fermentation pit and gas holder are combined into one brick structure, which makes it cheaper and easier to construct and maintain a bio-gas plant with a village's own resources. Although the initial versions suffered badly from gas leakages, PRAD workers now claim that their improved design is not only as efficient as the KVIC design but also half its cost. About 110 plants have been built in Uttar Pradesh and Rajasthan, and PRAD is organising training courses for constructing these plants, now called Janata (People's) Bio-gas Plants.

KVIC is meanwhile seriously evaluating its own new design in which both the digester and the gas holder are made of ferrocement. The digester is made up of a series of modular ferrocement rings which fit into each other, and the ferrocement gas holder slides into the digester. The farmer, has nothing to do but dig a suitable size pit. Some 100 such experimental plants have been set up and they are reported to be operating very satisfactorily. The ferrocement plants are about 20% cheaper than the conventional plants, but still slightly dearer than that of the Janata plants.

A hybrid design, incorporating a ferrocement gas holder with a conventional brick and mortar digester has been developed by the Structural Engineering Research Centre at Roorkee, and several voluntary groups are also working on new bio-gas plant designs. Mr J.C. Kapur of Kapur Solar Farms in New Delhi has developed a combined system of solar water heaters and bio-gas plants. The output of bio-gas plants falls drastically during the winter months, when minimum temperatures in northern India drop to near zero, and these are the months when more cooking energy is required. Solar heated water can keep the temperature in the digester at an optimum level and thus maintain the gas output. The bio-gas plant

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provides energy for cooking, to generate electricity for lighting, and also to take over from the solar plant for air conditioning when solar intensity is low. Kapur foresees a totally automatic interactive system.

Searching for new feedstocks

Simultaneously, several efforts are being made in various agricultural universities and research centres to find new feedstocks for bio-gas plants. The KVIC itself is experimenting with plants that use agricultural wastes (straw from wheat or rice, for example) with not more than 5% dung. Another possibility is water hyacinth, a weed that grows prolifically in various parts of India. The PRAD workers have already found that a mixture of water hyacinth and dung in the ratio of 2:3, gives a gas output increased by 40-50% and also a slurry richer in nitrogen, phosphates and potash. The KVIC is also experimenting with bio-gas plants which use urine as a feedstock: in some states the villagers are beginning to show less aversion to using human wastes in bio-gas plants.

The KVIC proposes to set up five regional bio-gas experimental centres to try out various types of bio-gas plant with a range of feedstocks in different agro-climatic, socio-economic and resource conditions. This type of research should help to identify the best designs for different regions.

But despite this vast programme, bio-gas researchers continue to be treated as second-class citizens in energy research. Work at the Gobar Gas Research Station of PRAD in Ajitmal, for instance, is treated by research workers as an interim arrangement until they find a better job. Researchers still receive low salaries and suffer from job insecurity, conditions under which no Indian nuclear researcher, for instance, would ever work. Successive Prime Ministers have stated that bio-gas is a matter of national interest, and India still needs to implement fully this good intention. □

news and views

Earth's cratering rate

from David W. Hughes

THE hysteria over Skylab's return to Earth and the unease engendered by the fact that about 600 of the 4,500 or so orbiting pieces of space hardware re-enter the atmosphere every year pales into insignificance against the shock that would follow the impact of an asteroid and the subsequent cratering event. How often are these impact craters produced? Well, a body capable of forming a crater with a diameter over 1 km hits the Earth every 1,400 years. Craters of 10 km diameter and more can be produced every 140,000 years assuming the impacting object hits land and not sea. These figures come from two reviews of the subject by R.A.F. Grieve and P.B. Robertson and Grieve and M.R. Dence of the Canadian Department of Energy, Mines and Resources and published in a recent issue of *Icarus* (38, 212; 230; 1979).

Terrestrial impact craters are fairly common and Fig. 1 shows their world wide distribution. Twelve of the structures shown in Fig. 1 are classified as proven meteorite craters because meteorite fragments have been found in their vicinity. The remaining 75 are 'probable' meteorite craters recognised as such because the target rocks have suffered shock metamorphic effects. It is obvious from the uneven distribution that the probability of their preservation and detection varies considerably from place to place. The majority occur in North America and Europe, areas with relatively stable geology and also with an active population of crater searchers. Erosion and geological processes progressively erase craters. For example a 20 km structure is only recognisable as a probable impact crater for a maximum of 600 million years whereas a 10 km structure usually has a lifetime of only 300 million years.

Morphologically there are simple bowl-like craters with an uplifted and overturned rim and complex craters with uplifted central peaks and slumped or depressed rims. Normally the simple craters have diameters of less than 4 km, the larger ones



Fig. 1 The world wide distribution of impact craters (after Grieve and Robertson).

being complex.

Plotting the number of craters, N , which have diameters D greater than D as a function of D it can be seen that $N \propto D^{-2}$ for D larger than 20 km. The curve departs from linearity below this size because small craters have a shorter lifetime and are less likely to be recognised. It is highly probable that the $N \propto D^{-2}$ relationship actually extends down to crater diameters of about 1 km. Martian craters obey an $N \propto D^{-2}$ law. Large lunar craters lie between $N \propto D^{-1.8}$ and $N \propto D^{-2}$. This law can also be justified by combining the mass distribution of the causative asteroids (the number of asteroids more massive than M is proportional to $M^{-0.6}$) with the relationship between the crater diameter and the kinetic energy, E , of the impacting body ($D \propto E^{0.29}$).

To calculate the cratering rate one needs ideally a large area of the Earth's landmass which has a single age of origin, preferably as old as possible, and which has undergone a minimal and uniform level of subsequent modifying geological activity. Two areas approximate to this. The first is the North American stable region that lies between the Rockies and the Appalachians and the Quachita and the Arctic. The area is 1.25×10^7 km² and the exposure age varies from 450 million years in the north to about 300 million years in the south. It contains 33 impact structures over 1 km in

diameter, the estimated production rate being $(0.36 \pm 0.1) \times 10^{-14}$ km⁻² yr⁻¹ for craters with $D > 1$ km. The second area is the Northern European plain lying between the Scandinavian Caledonides, the Urals, the Carpathians and the Caucasus. Here there are 20 craters with $D > 1$ km. Over an area of 4.5×10^6 km². As with North America the exposure age varies from 450 million years in the north to 300 million years in the south resulting in a production rate of $(0.35 \pm 0.13) \times 10^{-14}$ km⁻² yr⁻¹ for craters with $D > 20$ km. Combining these two production rates gives the equation

$$\log_{10} \phi = -11.85 - 2 \log_{10} D \quad (1)$$

where ϕ is the production rate (km⁻² yr⁻¹) of craters with diameters greater than D km. This equation is definitely valid down to diameters of 20 km and probably valid down to diameters of 1 km. The results can also be interpreted as indicating that this cratering rate has remained reasonably constant over the last 3.4×10^9 yr. Equation (1) shows that, assuming the Earth's surface was all land, a 1 km crater would be produced every 1400 yr and a 10 km crater every 140,000 yr.

We must be thankful that two-thirds of the globe is covered by water and, of the remaining land mass, the frozen wastes, jungles, deserts and mountains account for over 90% of the area. □

David W. Hughes is a Lecturer in the Department of Physics, University of Sheffield.

Gene mapping at the crossroads

by Mary Lindley

THE human gene map is said to be poised for rapid expansion, during the next few years, as the techniques of molecular biology and immunology take firm root in this traditional domain of geneticists and cell biologists. At a recent reunion in Edinburgh*, 150 of the aficionados agreed that they have now identified the positions of about 350 genes on the human chromosomes. Although the current rate of increase is a mere two or three genes per month, the atmosphere was full of confidence that this will accelerate rapidly, making a complete human gene map a possibility within the lifetime of some of the younger members of the gene mapping fraternity.

One of the most promising strategies involves the combination of some of the familiar tools of cytogenetics with techniques developed to investigate the fine structure of both prokaryotic and eukaryotic genes. By such methods the β -globin gene, already believed to be on chromosome 11, has been localised more precisely by D. Housman (Massachusetts Institute of Technology) and colleagues. They used cells from hybrid clones each of which contained the Chinese hamster genome together with a section of human chromosome 11. To identify the section carrying the β -globin gene they extracted the DNA from cells of each clone, treated it with a restriction endonuclease and separated the resulting fragments on agarose gel. The separated fragments were transferred onto nitrocellulose filters and hybridised to a radioactively labelled cloned human β -globin DNA probe. The pattern of hybridisation revealed by autoradiography showed that the gene for human β -globin is carried on a section of the short arm of chromosome 11. Because the gene for β -globin is known to be linked to those for γ , δ , and ϵ globins, all four genes must be carried on this chromosomal region.

By a similar procedure, B.R. Migeon and colleagues (Johns Hopkins University School of Medicine, Baltimore) have used hybrid cells containing the mouse genome and a human chromosome resulting from a translocation between chromosome 11 and the X chromosome (*Proc. natn. Acad. Sci. U.S.A.* in the press). They were able to select for the presence or absence of the human chromosome because it carried a functioning gene for the enzyme hypoxanthine guanine phosphoribosyl transferase, which the mouse cells lacked. These experiments have further confirmed that the β and δ -globin genes are carried on human chromosome 11, and show that they are not on the segment involved in the

translocation.

The application of 'restriction mapping' to increasing numbers of genes now depends on the rate at which cloned probes can be produced and characterised. F.H. Ruddle (Yale University) predicted that restriction mapping will become the predominant method for mapping genes, and that the major part of the human gene map could be known by the end of this century.

Speed, convenience and scope for fine resolution should give restriction mapping an advantage over *in situ* hybridisation, which has recently revealed further information about the arrangement of the histone genes on chromosome 7. Using labelled probes of complementary RNA, prepared from cloned sea urchin genes, D.M. Steffensen (University of Illinois, Urbana) and J.J. Yunis and M.E. Chandler (University of Minnesota Medical School) have both localised the five human histone genes to the long arm of this chromosome. Their results, however, do not indicate precisely the same positions, with the latter group placing the cluster of genes nearer to the end of the long arm (Chandler *et al. Science*, in the press). The difference may be due to the difficulty of interpreting the pattern of silver grains in the final, autoradiographic stage of this procedure.

Another promising line of enquiry for the future is provided by cell surface molecules which can be recognised by biochemical and immunological techniques and correlated with the presence of particular chromosomes. A. de la Chapelle (Folkhals Institute of Genetics, Helsinki) and colleagues have followed up previous studies which indicated an association between monosomy for chromosome 7 and defective granulocyte chemotaxis (Rutuu *et al. Nature* 265, 146; 1977). Treating the defective monosomic granulocytes with a radioactive label specific for glycoproteins, the Helsinki group has shown that these blood cells have much less than the normal amount of one particular glycoprotein. This suggests that a gene on chromosome 7 codes for a glycoprotein associated with chemotaxis. The same chromosome may also be involved in the expression of receptors for epidermal growth factor, according to N. Shimizu and colleagues (University of Arizona, Tucson). They have correlated the presence of this chromosome in hybrid cells with the ability to bind epidermal growth factor.

Indirect immunofluorescence, used by C.K. Eun and H.P. Klinger (Albert Einstein College of Medicine, New York), has suggested that the expression of fibronectin on cell surfaces is affected by chromosome 11. This contrasts with the suggestion of Owerbach *et al. (Proc. natn. Acad. Sci. U.S.A.* 75, 5640; 1978), who

labelled surface proteins by lactoperoxidase-catalysed iodination and correlated the presence of fibronectin with chromosome 8. The disagreement arises again in the results of a similar study by M. Smith (Mount Sinai School of Medicine, New York) and colleagues who found that hybrid cells could be positive for fibronectin while lacking glutathione reductase which is associated with chromosome 8. The genetics of fibronectin it seems may be complex, and possibly may involve genes on various chromosomes. On the other hand, the discrepancy may be resolved by the application of more refined techniques.

Another promising area is represented by two reports of genes that influence the expression of oncornaviruses. Using human-mouse cell hybrids, C.E. Wright and T.B. Shows (Roswell Park Memorial Institute, Buffalo) have found a gene on human chromosome 11 that induces the expression of a mouse xenotropic oncornavirus and another on chromosome 15 involved in the induction of endogenous mouse N-tropic oncornavirus. They call these genes *BVIX* and *BVIN*, respectively, and have found that they are carried on human chromosomes homologous to mouse chromosome regions that also carry genes affecting the expression of oncornaviruses.

This suggestion of genetic conservation during evolution is just one of many examples that are emerging as gene maps are developed for different mammalian species. As well as those of mouse, rabbit and various primates, genes are now being assigned to the chromosomes of cow, sheep, pig, cat, dog and horse. As would be expected, these are providing further evidence of the conservation of the X chromosome during evolution. It is also becoming increasingly clear that other linkage groups have been strongly conserved, especially between the great apes and man. Yunis and Chandler, for example, have extended their *in situ* hybridisation study to chimpanzee, gorilla and orangutan and localised the histone genes on homologous regions of the counterparts of human chromosome 7.

Similarly J.J. Garver (State University, Leiden) and colleagues have localised the major histocompatibility complexes of chimpanzee and rhesus monkey on a chromosome homologous with human chromosome 6, which carries the HLA complex. P.A. Lalley and colleagues (Oak Ridge National Laboratory) have found that some genes on human chromosomes 1 and 6 are correspondingly linked in the baboon, indicating conservation. On the other hand, they interpret their study of the baboon counterpart of human chromosome 2, as a sign that extensive chromosomal rearrangements have occurred during the evolution of this chromosome. Many more such insights should come as the various gene maps develop. □

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*The fifth International Workshop on Human Gene Mapping was held in Edinburgh on 9-13 July 1979. It was organised by H.J. Evans, MRC Clinical and Population Cytogenetics Unit, Edinburgh. The abstracts and committee reports will be published in *Cytogenetics and Cell Genetics*.

EIGHT years ago Morgan (*Nature* 230, 42; 1971) proposed the existence of hotspots, which he envisaged as manifestations at the Earth's surface of narrow plumes of hot mantle material rising from below. Ever since these supposed lithospheric penetrations have been a perpetual source of controversy. Do they exist at all? And if they do, how many are there — a few, or tens, or even hundreds? Are they really linked to deep mantle plumes? If so, are the plume positions laterally fixed in the mantle or do they (and hence the hotspots) move relative to each other? Do hotspots-plumes really account for the phenomena (Hawaiian volcanism, for example) often attributed to them? And so are linear volcanic age trends (the Hawaiian-Emperor chain, for example), hotspot traces, resulting from the motion of tectonic plates over (stationary?) plumes beneath?

Nothing like a consensus has yet emerged on any of these questions. Which makes it all the more fascinating to observe that Thiessen *et al.* (*Geology* 7, 263; 1979) have had the temerity to superimpose this set of controversies on another uncertainty, namely, the mobility/immobility of Africa. A few years ago Minster *et al.* (*Geophys. J.* 36, 541; 1974) concluded that for the past 10 million years Africa has been moving at the rate of about 2 cm a year. Burke and Wilson (*Nature*, 239, 397; 1972), on the other hand, have taken the view that for the past 25 million years or so Africa has been stationary with respect to the underlying mantle; and there is much to be said for this suggestion. Palaeomagnetic data appear to rule out any major latitude changes during this period; and about 25 million years ago there was a dramatic increase in African volcanism, implying a continent that had come to rest relative to its underlying volcanic sources.

Of course, to anyone strongly committed to the existence of mantle

Hotspots and African mantle

from Peter J. Smith

plumes, African volcanism and African hotspots are synonymous; and to that extent Burke and his colleagues made the hotspot-immobility connection right from the start. According to this vision, the increase in African volcanism about 25 million years ago and the continued high level of volcanic activity since then simply reflect the relative ease with which mantle plumes may pierce stationary, as opposed to moving, lithosphere. Then regions of high relief/elevation without associated volcanisms are regarded as nothing less than incomplete hotspots, or highspots, resulting from the same underlying processes as the hotspots proper. Thus Africa's distinctive basin and swell topography, where the swells are seen as hotspots/highspots, becomes a natural consequence of the continent's immobility.

Those who remain unconvinced about the reality of mantle plumes, or who accept the existence of only a few of them, will find this proliferation of hotspots and quasi-hotspots outrageous. Nevertheless, it is interesting to see how Thiessen and his colleagues (who, incidentally, include Burke) combine hotspots and a stationary Africa into a consistent picture and then go on to develop the model in terms of its implication for the underlying mantle. For accepting that Africa is stationary (that is, that primary convection beneath the continent is suppressed) and that all volcanism and uplift is essentially hotspot activity, it becomes possible to speculate

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on the nature of the plume convection system beneath.

As Thiessen (R.) *et al.* see it, there are some 30–40 hotspots/highspots within Africa's boundaries. That is, there are some 30–40 narrow zones of upwelling mantle material; and as what comes up must go down (well, some of it), there will be downwelling of mantle material approximately midway between hotspots. For a random arrangement of hotspots, this downwelling must occur along the edges of polygons (as viewed in plan), one of which surrounds each hotspot. In other words, there will be a pattern of polygonal convection cells, as seen from above, such that any points within a particular cell will be nearer to that cell's hotspot than to any other hotspot.

The construction of such polygons in a mathematical sense was first carried out by Thiessen (A.H.) in 1911 in connection with an analysis of rainfall data, whence, as widely used in computer cartography, they have come to be known as Thiessen polygons. Of more geophysical interest, however, is the fact that Richter and Parsons (*J. geophys. Res.* 80, 2529; 1975) were able to generate polygonal cells in laboratory convection experiments involving oil layers heated from below in cases where the upper layer was stationary. Moreover, assuming the real-Earth plume cells to extend down only to the 700 km seismic discontinuity, the experimental and African polygons had the same width-depth ratios. Thus laboratory work and observationally-based hypothesis combine nicely to produce a consistent picture.

Except that Morgan's original plumes were deep-mantle plumes, possibly originating as far down as the core-mantle interface, whereas Thiessen's plumes appear to be an upper-mantle phenomenon only. Something seems to have been lost here without acknowledgment.

Atomic spectroscopy at Orsay

from Anne Thorne

Do you prefer to believe in 'action at a distance' or in 'hidden variables in quantum mechanics'? Put another way, if quantum mechanics affords a complete description of a system, it seems to be necessary to reject the idea of local causality — that is, the idea that the causes of effects are to be found inside the relevant backward light cone. Reviewing the present status of this old dilemma at a recent conference* J.S. Bell (CERN, Geneva) described a *Gedankexperiment* in which an excited atom in a state A undergoes the radiative cascade $A \rightarrow B \rightarrow C$,

emitting a photon at each stage, where A and C have zero angular momentum and the same parity. Taking the particular case that the two photons are emitted in opposite directions, then if one is linearly polarised so also must the other be, with the electric vector in the same direction. Hence information from a perfectly efficient linear polariser on one side gives information about the signal from a second perfectly efficient linear polariser on the other side. Bell described attempts to translate *Gedanken* into reality, including a variant in which the angle between the polarisers is changed while the photons are actually in flight. Although the results so far seem to favour quantum mechanics rather than 'local causality', a sceptic can

still find refuge in the allowances made for detector inefficiencies, finite acceptance angles, and so on. Attempts are now being made to close these loopholes and obtain what is expected to be incontrovertible evidence in favour of quantum mechanics. Meanwhile, most of us will continue to ignore the challenge of this intriguing problem by adopting the first of the three options Bell put forward — "Don't think about it at all!"

On the same day, nicely redressing the balance between the conceptual and the practical, H. Welling (Institute of Applied Physics, Hannover) discussed important

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*The 11th Conference of the European Group for Atomic Spectroscopy was held at Orsay on 10–13 July. The Organising Secretary was Professor B. Cagnac, ENS.

new developments in tunable lasers. The spectral region over which tunable lasers can be operated is extending rapidly into both the ultraviolet and the infrared. Welling concentrated mainly on the solid state F-centre lasers for the infrared. In particular, the F_A and F_B colour centre lasers, made by doping crystals of the heavier alkali halides with lithium and sodium respectively, cover between them nearly all the spectral region between 0.8 and 1.9 μm . The power output is in the region of 100mW, with the laser running over two or three modes (2-3 GHz), but Welling suggested that the final resolution attainable should be of the order of 100kHz.

C. Cohen-Tannoudji (ENS, Paris) discussed several intriguing experiments with atoms in intense laser fields. For example, a gas can be radiatively cooled by momentum transfer between atoms and laser radiation detuned slightly from the absorption line centre, so that photons are absorbed only by atoms travelling towards them. The cooling after many such collisions is considerable: an effective temperature of as low as 0.2 K was mentioned. As another example, the energy shift of atoms in intense laser fields can be exploited for Doppler-free spectroscopy by choosing the intensity and the detuning of the laser to compensate the Doppler shift for atoms travelling in the forward direction. The Doppler shift for atoms travelling backwards is correspondingly increased resulting in a high degree of forward-backward asymmetry in the absorption line. Moreover, interpreting this same intensity shift as a change in potential, it can be seen that a high intensity gradient, as in a beam focused to a narrow waist, implies a force on the atom constraining it to the high field region. Two crossed lasers can thus provide a trap for neutral atoms, in which it should, among other things, be possible to observe a single atom over a finite period. This opens up the possibility of performing on neutral atoms the type of experiment (lifetime of metastable states, high precision hyperfine structure) that has recently been achieved with ion traps.

One particular set of experiments may be of interest to physicists outside the field of spectroscopy. These concern the measurement of the hyperfine structure of the artificial atoms muonium, μ^+e^- , and muonic helium, $\alpha\mu^+e^-$, a joint project between Heidelberg and Yale Universities, conducted at Los Alamos Scientific Laboratory. In the case of muonium, the ratio of the muon and proton magnetic moments and the hyperfine splitting of the ground state are independently determined, and comparison of the latter with the theoretical value represents a clear test of the QED corrections. The muonic helium atom was aptly described as a "set of Dutch doll hydrogen atoms": the system electron plus pseudonucleus $\alpha\mu^+$ constitutes the outer hydrogen-like atom and the system $\mu^+ + \alpha$ the inner one. □

International prostaglandin conference

from Marie Foegh

ONE of the most exciting discoveries announced at the recent International Prostaglandin Conference* was the structure of slow-reacting substance (SRS) which has been determined by B. Samuelsson and colleagues at the Karolinska Institute, Stockholm (see Fig. 1). SRS-A (slow reacting substance of anaphylaxis), is one of the several mediators of anaphylaxis, and is of particular interest because it is synthesised *de novo* as a consequence of anaphylactic challenge and considered to be a major cause of bronchospasm during asthmatic attack. Despite much effort, the structure remained unsolved for many years.

SRS has now been identified as a novel cysteinyl derivative of arachidonic acid with a hydroxyl group at C-5 and three conjugated ($\Delta^7, 9, 11$) and one isolated (Δ^{14}) double bond. The amino acid (cysteine) is linked as a thioether to C-6. These findings indicate the importance of the lipoxygenase pathway in its synthesis. Samuelsson introduced the term 'leukotriene' for these metabolites which are non-cyclised C_{20} carboxyl acids with one or two oxygen substitutes and conjugated double bonds. E.J. Corey (Harvard University), described routes of total synthesis to leukotriene.

SRS is a polar lipid-like substance. Arachidonic acid had previously been suggested as a precursor of SRS for various reasons and this is confirmed by the structure. The clinical implications of the newly-determined structure of SRS are apparent in immune responses of the immediate type. The non-steroidal anti-inflammatory drugs, such as aspirin, enhance SRS-A production *in vitro* by inhibiting the cyclo-oxygenase pathways of arachidonic acid metabolism. Aspirin is therefore expected to enhance SRS-A production *in vivo* and that may explain aspirin-sensitive asthma — an immediate anaphylactic reaction to aspirin and aspirin-like drugs observed in some asthmatic patients.

In clinical experience this pathological response occurs mainly in females. Falliers (*J. Allergy & clin. Immunol.* 52, 141; 1973) has shown in a follow-up of 1,298 asthmatic patients who developed their asthma in childhood, that 1% of male patients and 4% of female patients had aspirin-sensitive asthma. These sex-differences may be related to the manner in which arachidonic acid is anabolised and catabolised. Sex differences in both platelet and vascular responses to arachidonic acid in animals were reported by P. Ramwell (Georgetown University, Washington, DC) where the

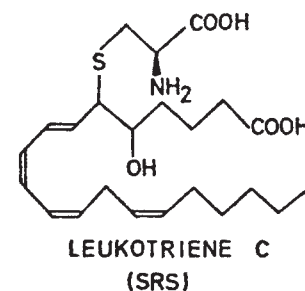


Fig. 1

male is more responsive than the female.

J. Vane (Wellcome Research Laboratories, Beckenham, UK) presented further evidence for the hypothesis that the bleeding defect in the Greenland Eskimo is caused by an increased production of prostacyclin I_3 by vascular endothelium which inhibits platelet aggregation. An increase in production of a prostacyclin-like substance from arterial rings incubated with $\Delta^5, 8, 11, 14, 17$ eicosapentenoic acid has been demonstrated. Because of their fish diet, Greenland Eskimos have high plasma levels of eicosapentenoic acid which is the precursor of the three series of endoperoxides — prostaglandins, thromboxane and prostacyclin. In contrast to thromboxane A_2 produced from arachidonic acid, thromboxane A_3 has only a weak aggregating effect on platelets, while PGI_3 is a potent anti-aggregation substance. However, P. Needleman *et al.* (Washington University, St Louis), suggested an alternative explanation based on their observations that mammalian tissues (except for ram seminal vesicles) cyclised eicosapentenoic acid very poorly and that moreover, this acid had the same affinity as arachidonic acid for the cyclo-oxygenase enzyme. Furthermore, eicosapentenoic acid inhibited the cyclisation of arachidonate. Tissues and organs readily incorporated isotopic eicosapentenoic acid, like arachidonic acid, and it was as easily released as arachidonic acid following bradykinin stimulation. Needleman suggested that the bleeding defect in the Greenland Eskimo may be due to a decreased tendency for platelets to make thromboxane A_2 as a result of the presence of high levels of eicosapentenoic acid in plasma and platelet membrane inhibiting the cyclisation of arachidonate. Whatever the explanation, these observations raise the possibility of therapeutic intervention by diet.

Many papers dealt with the newly discovered prostacyclin. Because of its vasodilator and platelet anti-aggregating properties it has already been tested for treatment of peripheral artery diseases, and for its usefulness during haemoperfusion, haemodialysis and heart valve replacement.

In obstetrics, there have been further developments of prostaglandins of the E

*The International Prostaglandin Conference was held in Washington DC on 27-31 May, and organised by P. Ramwell, B. Samuelsson, and R. Paoletti. The proceedings of the Conference will be available from Raven Press, New York.

series, where 16-phenoxytetranor-PGE₂ methyl-sulphonyl-amide (Schering, Pfizer), 16, 16-dimethyl *trans*-Δ²PGE₁ methylester (ONO) and 9-deoxy-16, 16-dimethyl-9-methylene PGE₂ (Upjohn) have been tested for use in both early (including menstrual regulation) and late abortion. The new compounds seem to produce fewer gastro-intestinal side effects and can be given intramuscularly, vaginally or orally. M. Bygdeman (Karolinska Institute, Stockholm) reported successful abortions in 10 of 12 women in the second trimester after oral doses of 25 mg of 9-deoxy-16, 16-dimethyl-9-methylene PGE₂ when given every third hour; the mean abortion time was 22 h.

The conference illustrated the general applicability of prostaglandins and related compounds to all aspects of medicine. Just as prostacyclin and thromboxane synthetase inhibitors are now being developed as anti-coagulants one can look forward to major efforts to develop specific lipoxygenase inhibitors and leukotriene C antagonists for clinical use. The development of radioimmunoassays for leukotriene C should be of considerable diagnostic value in immunological diseases. □

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Sugars and intracellular recognition

from M. Geisow

STUDIES of the biosynthesis and structure of membrane glycoproteins have reached maturity in the past few years. The long-suspected role of sugars in cell-surface recognition processes has been established with the identification of specific receptors. However, the reason for the glycosylation of many soluble secreted proteins (plasma proteins and hormones for example) is still not at all clear. Do such conjugated sugars have analogous extracellular recognition functions? In 1965, while considering the selective export of secreted proteins, Eylar (*J. theoret. Biol.* 10, 89) hypothesised that conjugated sugars formed part of a primitive mechanism of export from cells. This early idea was right to the extent that it predicted the existence of an export 'signal', but wrong in the sense that this signal has turned out to be a peptide rather than an oligosaccharide (see *News and Views* 272, 308; 1978). In eukaryotic cells, newly synthesised proteins entering the lumen of the rough endoplasmic reticulum (RER) by virtue of this signal peptide must

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Yes, NGF is an enzyme

IN a recent article in the News and Views section of this Journal, P. Calissano and R. Levi-Montalcini have questioned the validity of our work on the proteolytic activity of nerve growth factor (NGF), and in particular, its ability to activate plasminogen¹. Because their article contains misinformation, and because several of our papers on the points raised by Calissano and Levi-Montalcini were overlooked, we wish to respond as follows.

In 1977 we observed that crude homogenates of mouse submandibular glands contain multiple molecular-weight forms of NGF². The largest of these, with mass 116,000, was completely purified² and later shown to be a specific proteolytic enzyme that can activate plasminogen³. It is also the predominant form of NGF which is secreted in high concentrations in mouse saliva⁴. Calissano and Levi-Montalcini state that this form of NGF "... exhibits a protease activity with an almost evanescent degree of specificity for plasminogen ...". Yet, as shown in Figs 1, 3, and 4 in reference 3 there is nothing about the proteolytic activity of this species of NGF which indicates that it is transient. Rather, the enzyme activity is real, persistent, and highly specific. NGF is indeed much less active than urokinase in activating plasminogen, and we clearly pointed that out. We also stated that plasminogen might not be the natural (physiological) substrate for NGF — only that it was susceptible to activation by this protease³.

With regard to the possibility that the protease associated with our NGF preparations might be a nonspecific contaminant¹, Fig. 4 in reference 3 presents strong evidence that this is not the case. This figure shows that when our preparations of the 116,000 molecular-weight form of NGF are examined by gel electrophoresis, all of the protein, all of the enzyme activity, and all of the immunoassayable NGF activity migrate with identical electrophoretic mobilities — as a single component.

More recent studies have revealed that this form of NGF exhibits some unusual features which are perhaps even more interesting than its plasminogen-activating property. For example, we have now shown that NGF, as isolated from mouse salivary glands, exists as a

zymogen and further, that this zymogen is converted to fully active enzyme by autocatalytic self-activation⁵. The zymogen itself is largely, if not entirely, enzymically inactive. Only after autocatalytic activation is full enzyme activity expressed⁵. This finding is probably the strongest evidence that the protease activity of NGF is specific and that it does not stem from a contaminant.

With regard to the comment of Calissano and Levi-Montalcini that the NGF activity might be "stuck" nonspecifically to other proteins present in the salivary gland, we must point out that if this is so, then it is "stuck" extremely tightly, it is "stuck" in constant ratio from preparation to preparation, and it is also "stuck" as it is naturally secreted in saliva⁴. All of this goes to the heart of an elementary problem of protein chemistry; namely, what constitutes the definition of a true molecular species. All of our results outlined above suggest that the NGF-zymogen is such a species, and we know of no published evidence which indicates otherwise.

Finally, we should like to point out that of the several (at least six) forms of NGF present in salivary gland extracts, only the 116,000 molecular weight protein is stable³. All of the other forms are degradation products of this single species². This includes the 2.5S-NGF (molecular weight 26,000) referred to by Calissano and Levi-Montalcini¹. In fact, 2.5S-NGF accounts for less than 10% of the total NGF found in the salivary gland, and this minor component is the one which has been used in most experiments on NGF over the past twelve years.

The proteolytic activity of the 116,000 molecular weight form of NGF is a real property of this protein, and this fact, as well as the autocatalytic activation reaction, is going to have to be taken into account before the true physiological function of NGF can be understood.

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1. Calissano, P. & Levi-Montalcini, R. *Nature, News & Views* 280, 359 (1979).
2. Young, M., Saide, J.D., Murphy, R.A. & Blanchard, M.H. *Biochemistry*, 17, 1490 (1978).
3. Orenstein, N.S., Dvorak, H.F., Blanchard, M.H. & Young, M. *Proc. natn. Acad. Sci. U.S.A.* 75, 6597 (1978).
4. Murphy, R.A., Saide, J.D., Blanchard, M.H. & Young, M. *Proc. natn. Acad. Sci. U.S.A.* 74, 2672 (1977).
5. Young, M. *Biochemistry* 18, 3050 (1979).

nevertheless possess further chemical or structural attributes which will determine their ultimate fate. For example, complex enzymic processing follows the biosynthesis of hormone precursors like ACTH/endorphin (Mains *et al.* *Proc. natn. Acad. Sci. U.S.A.* 74, 3014; 1977). Furthermore, molecules within the ER/Golgi system, whether processed or

not, need to be packaged in a different manner according to whether they are lysosomal, secreted or membrane proteins. Recent experimental work suggests the participation of conjugated carbohydrate in these intracellular recognition processes.

Melchers (*Biochemistry* 12, 1471; 1973) found that the glycosylation inhibitor 2-deoxy-D-glucose prevented migration of

newly-synthesised IgG1 from RER to smooth ER, although it is known that this sugar acts at a number of cellular sites. Subsequent studies have taken advantage of the more specific action of tunicamycin. This drug inhibits the attachment of N-acetyl glucosamine to a lipid carrier, dolichol phosphate, blocking the so called 'core glycosylation' of glycoproteins. Hickman *et al.* (*J. biol. Chem.* **252**, 4402; 1977) supported Melcher's finding when they reported that secretion of newly-synthesised IgA and IgE from plasma cells was blocked by tunicamycin. The release of λ light chains (normally not glycosylated) was unaffected and there was only minor perturbation of protein synthesis.

Although intact, non-glycosylated immunoglobulins can be detected in quantity in tunicamycin-treated cells, other glycoproteins seem to be degraded intracellularly. Schwarz *et al.* (*J. Virol.* **19**, 782; 1977) found that tunicamycin prevented the formation of mature Semliki Forest virus and fowl plague virus (FPV). At least one of the virion proteins (the sugar-free precursors of FPV haemagglutinin) was subject to proteolysis, since the specific trypsin inhibitor TLCK was needed to detect the presence of this characteristic virus protein. Analogous intracellular proteolysis of non-glycosylated fibronectin (the extracellular matrix-forming protein) was reported by Olden *et al.* (*Cell* **13**, 461; 1978).

Related, but more subtle consequences of blocking glycosylation have been described for two soluble secreted proteins. Duksin and Bornstein (*J. biol. Chem.* **252**, 955; 1977) found that non-glycosylated procollagen was secreted normally by fibroblasts, but that a step in its extracellular conversion to collagen (removal of the 3-chain carboxyl-terminal extension) was impaired. Since the non-glycosylated procollagen was processed normally by extracts from untreated fibroblasts, the impairment was probably due to the absence of cosecreted processing enzyme. Loh and Gainer (*FEBS Lett.* **96**, 269; 1978) found that the biosynthesis of ACTH/endorphin precursor in frog pituitary was unaffected by tunicamycin. However, chase experiments indicated that the subsequent proteolytic processing of this precursor was quite different in tunicamycin-treated glands. The alterations in processing particularly affected the ACTH region of the molecule, which is close to a site of sugar attachment. The processed products were recovered in poor yield, suggesting that the precursor was exposed to nonspecific fragmentation.

Not all proteins derived from ER membrane-bound ribosomes are so markedly affected by tunicamycin. Leavitt *et al.* (*J. biol. Chem.* **252**, 9018; 1977) used tunicamycin to prevent the glycosylation of vesicular stomatitis virus and Sindbis virus proteins. The non-glycosylated versions were metabolically stable, but the proteins remained 'cryptic' — they could not be

detected on the host cell surface at any stage during the process of infection. Moreover, the sugar-free virion proteins were insoluble in detergent, unlike the normal glycoproteins. The authors speculated that the altered intracellular migration and physical chemical properties of the proteins stemmed from an altered conformation of the non-glycosylated polypeptides.

Semliki Forest virus proteins also remain cryptic in tunicamycin-treated cells (Garoff & Schwarz *Nature* **274**, 487; 1978). In this case gross perturbation of conformation is unlikely, since the non-glycosylated proteins are processed correctly by host cell proteases and inserted correctly into the ER membrane. Ovalbumin is one soluble secreted protein whose biosynthesis and release appears to be unaffected by tunicamycin-treated hen oviduct cells (Keller & Swank *Biophys. Biochem. Res. Commun.* **85**, 72; 1978).

An unattributable item of biochemical wisdom alleges that attachment of sugar moieties to glycoproteins confers a degree of immunity to proteases and that this occurs as much by design as by chance. In the ACTH/endorphin system already referred to, the carbohydrate on the bovine precursor molecule (Nakanishi *et al.* *Nature* **278**, 423; 1979) is very probably close to such a proteolytic cleavage site. Absence of carbohydrate sterically placed to shield susceptible peptides might well explain the degradation of certain non-glycosylated molecules. In fact, this explanation is invoked by some authors. However, an extension of this argument seems worth mentioning here, since it

seems not to have previously been considered. By some device secretory material, processing enzymes and the complement of lysosomal enzymes must be segregated and separately packaged in the ER/Golgi system.

The majority, and possibly all, of the lysosomal proteins are glycoproteins. One in particular, β -glucuronidase, is known to be attached to the inner surface of the lysosomal membrane. Moreover, lysosomal proteins like β -glucuronidase in the extracellular space seem to be efficiently taken up into cells by way of a receptor-mediated event which recognises covalently-linked hexosephosphate (see *News and Views* **269**, 288; 1977).

These studies may be leading to the establishment of phosphorylhexose as an intracellular recognition signal. What happens to newly-synthesised, non-glycosylated lysosomal enzymes when secretory cells are treated with glycosylation inhibitors? Might they escape such a recognition system and enter an inappropriate compartment — a secretion granule for instance?

This line of argument leads full circle to Eylar's original hypothesis about the intracellular recognition role of conjugated sugars. Sugars do not specify export as such, but if they represent 'routing' signals within the ER/Golgi, then the diverse effects of tunicamycin might be more readily explained. Exploration of this idea will require experiments which trace normal and carbohydrate-free versions of membrane, secreted and lysosomal proteins through the cell. □

Electronic dimples explained

from P. V. E. McClintock

EARLIER this year M. Wanner and P. Leiderer reported (*Phys. Rev. Lett.* **42**, 315; 1979) that they had observed an intriguing two-dimensional crystal lattice of electron filled dimples at the interface of a phase separated ^3He - ^4He mixture. This unexpected experimental result, which was obtained at the Technical University of München, has now received a fairly complete explanation from H. Ikezi of Bell Laboratories, who describes his calculations in a recent issue of the same journal (*Phys. Rev. Lett.* **42**, 1688; 1979).

The two dimensional (2-D) layer of electrons which can be floated on the free surface of liquid helium has been studied in considerable detail: it is believed to take the form of a 2-D crystal lattice of the type first proposed by Wigner (*Trans. Faraday Soc.* **34**, 678; 1938). In practice an electric field must be applied, pushing the electrons on to the surface, and depressing the centre of the liquid slightly. The individual electrons, which are highly mobile parallel to the surface, are thus prevented from

spreading under their mutual Coulomb repulsion. If this electric field is made large enough (a few kV cm^{-1}), however, it is found experimentally that the surface eventually becomes unstable. The charge suddenly penetrates into the liquid, and it finally gets caught by the lower of the two electrodes providing the electric field. Studies of this instability provide information about the electron lattice and about surface waves on the liquid. Some quite detailed investigations have already been performed including, particularly, a beautiful cinematographic film of the onset of the phenomenon taken by Volodin, Khaikin and Edel'man (*J. exp. theor. Phys. Lett.* **26**, 543; 1977) and shown at the 15th International Conference on Low Temperature Physics at Grenoble in 1978.

Very similar considerations apply to the interfacial boundary which occurs where liquid ^3He floats on top of a dilute ^3He - ^4He solution, but this system has only recently come to be studied in any detail. Again, the

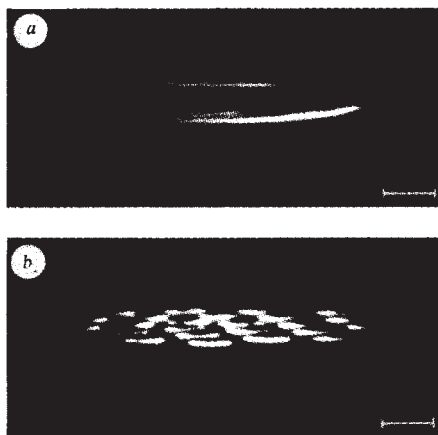


Fig. 1 Photographs of the deformation of the charged liquid $^3\text{He}/^4\text{He}$ interface at a temperature of 0.567 K, observed from below at an angle of 6° . *a*, For an electric field $E = 1120 \text{ V cm}^{-1}$ a single large circular indentation is produced, the elliptical bright lines indicating its rim. *b*, For $E = 1160 \text{ V cm}^{-1}$ the large indentation has spontaneously deformed into an array of much smaller circular dimples. The scale bar in each case in 1 mm.

interface can be charged with electrons and can then be depressed slightly by an electric field. Wanner and Leiderer's experimental arrangement allowed them to create ripples on the charged interface and to study the frequency-wavelength relationship (dispersion relation) using an optical technique. As anticipated theoretically they found that, for large electron densities and electric fields, the modes 'softened' — their frequencies become less for any given wavelength. This effect is a consequence of a reduction in the net restoring force for a displacement of the interface, caused by a rearrangement of electrons in the vicinity of the ripple. For very large fields, the frequency approaches zero and the whole arrangement becomes unstable, with charge breakthrough eventually occurring.

What Wanner and Leiderer found, however, was that a series of macroscopic dimples sometimes formed in fields quite close to that producing breakthrough. Their striking photographs are reproduced in Fig. 1. In (*a*) the surface is seen to be depressed in a single large diameter indentation; but, with quite a small

increase in the electric field, it spontaneously breaks up into a lattice of small indentations, as shown in (*b*). The indentations were typically 1 mm apart, forming what appeared to be a hexagonal lattice (although the oblique viewing angle made exact identification of the structure rather difficult). Since each dimple was calculated to contain about 10^6 electrons, it was not at all surprising that they should try to repel each other in order to minimise their Coulomb energy and, in so doing, form a macroscopic 2-D Wigner lattice. The authors added the intriguing thought that the electrons in each dimple would presumably also be arranged in a 2-D lattice, but with a lattice constant some three orders of magnitude smaller than that of the dimple lattice. What was not entirely obvious, however, was why any dimples should be produced in the first place.

Ikezi has now produced the answer. He has done so by extending a theoretical approach originally proposed by L.P. Gor'kov and D.M. Chernikova (*Soviet Phys. Dokl.* 21, 328; 1976), and has

developed a theory of the surface ripples which includes nonlinear terms. The latter turn out to be of great importance near the instability. Amongst the most interesting conclusions to emerge from his calculation is that, under certain conditions, his six coupled equations lead to solutions representing a surface profile which is both stable and static. It forms the lattice shown in Fig. 2. As far as one can tell, this is in excellent agreement with the dimple structure observed in the actual experiments (Fig. 1*b*).

Ikezi points out that both shear and compressional waves should propagate through the ^3He - ^4He dimple lattice; and that similar phenomena should also be observable on a free surface of superfluid ^4He . He predicts that the frequencies of these lattice waves should be typically a few Hertz, in which case they should not be too difficult to observe. It will be interesting to see whether he is right. $\square$

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Are supergroups the next step for electro-weak interactions?

from J.G. Taylor

IN THE past year impressive experimental confirmation has been obtained for the so-called electro-weak theory (EW) of Salam and Weinberg which unifies the electromagnetic and weak interactions under the gauge group $\text{SU}(2) \times \text{U}(1)$.

There is still a discrepancy between the Russian experiment on parity violation at Novosibirsk and an almost identical experiment conducted at Oxford¹; the former agrees remarkably well with the predictions of the Salam-Weinberg model, in complete disagreement with the null result obtained by the latter. In spite of this there is almost universal acceptance of the electro-weak theory.

However, there are still unsatisfactory features of the model. Thus the predicted intermediate vector bosons, (IVBs) of mass about 80 GeV, have still to be seen, though they should be created around 1981 at the pp facilities now being built. There is also considerable freedom in the mechanism as to how the IVBs get their masses; even the favoured one of spontaneous symmetry breaking (SSB) has great latitude in its detailed operation. Furthermore the EW theory is not really a unified theory; the

relative strengths of the interaction in the $\text{SU}(2)$ and $\text{U}(1)$ parts of the gauge group has to be obtained from experiment. The parameter measuring this strength, the Weinberg θ_w , has now experimentally settled down very close to the value $\theta_w = 30^\circ$ (ref.2) as given at the CERN EPS meeting in Geneva at the end of June.

Y. Ne'eman³, and D. Fairlie⁴ both independently noticed that the smallest group containing $\text{SU}(2) \times \text{U}(1)$ and which should require θ_w to be 30° is the supergroup $\text{SU}(2|1)$. This latter has as generators of its group algebra the four weak isospin and weak hypercharge generators of $\text{SU}(2)$ and $\text{U}(1)$ and in addition four anti-commuting generators. These additional generators have therefore the characteristic of fermions, and should correspond to spin half integer associated gauge fields.

Various attempts³⁻⁶ have been made to construct a satisfactory gauge field theory of $\text{SU}(2|1)$. None of these has been able to overcome completely the difficulty that since the symmetry group $\text{SU}(2|1)$ is not combined with the Poincaré group symmetry of space-time the gauge fields of $\text{SU}(2|1)$ will all be vector fields. If they have Fermi statistics, as should those which gauge the anti-commuting generators of $\text{SU}(2|1)$, they will therefore violate the spin-statistics theorem (which only allows integer spin fields to have commutation and not anti-commutation rules). Since the

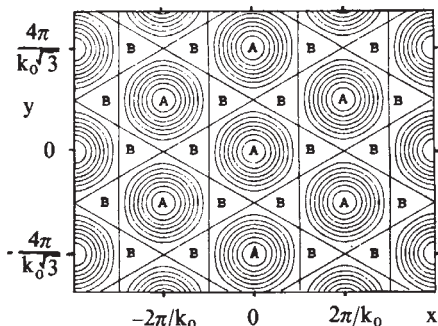


Fig. 2 Calculated surface profile for electric fields close to that of the instability: points A are the troughs and B are the peaks; and the contours indicate points of equal height. The troughs form a triangular lattice and the peaks a hexagonal one.

1. Barkov, L.M. *Proc. Int. Conf. High Energy Physics*, European Physical Society, Geneva, July 1979 (in the press).
2. Salam, A. *ibid.*
3. Ne'eman, Y. *Phys. Lett. B* 81, 190 (1979).
4. Fairlie, D. *Phys. Lett. B* 82, 97 (1979).
5. Dondi P. & Jarvis, *Phys. Lett. B* 84, 75 (1979).
6. Taylor, J.G. *Phys. Lett. B* 83, 331 (1979).
7. Ellis J. *Proc. Int. Conf. High Energy Physics*, European Physical Society, Geneva, July 1979 (in the press).

spin-statistics theorem can be deduced from very general field-theoretical principles such a violation is not to be accepted.

Some attempts^{3,5} have been made to avoid this difficulty by introducing anti-commuting supergroup parameters related to these odd generators. However the resulting dynamical theories seem to possess ghosts and other undesirable features. An alternative approach is to construct a dynamical theory which has classical fields so chosen as to be invariant only under infinitesimal variations of the group $SU(2|1)$. The condition of infinitesimal invariance leads to a sensible physical theory and also specifies the mechanism of SSB with no further latitude. Furthermore the theory avoids the problem of wrong-statistics gauge fields by no longer allowing arbitrary gauge invariance. Only the original electro-weak symmetry group $SU(2) \times U(1)$ is full gauged; the wrong-statistics gauge fields are not required.

Such an approach does not follow the tradition of gauging all possible symmetry groups. However it cannot be dismissed lightly since it does completely unify electromagnetism and weak interactions with Weinberg angle θ_w of 30° , as well as predicting the masses of the IVBs to have the same value as the original Weinberg-Salam theory. Furthermore it predicts an associated neutral scalar (Higgs) boson to exist with mass exactly twice that of the charged IVBs. Such a prediction may also be tested at the future pp facility, though with greater difficulty than for the IVBs due to the rather weak coupling of the Higgs boson to normal matter. Another prediction which can also be tested in about 1981 is that no lepton like the electron or muon can have mass larger than about 54 GeV.

The above theory can also be extended to include strong interactions. Quarks can be fitted into the four-dimensional representation of $SU(2|1)$ (as the leptons can into the three-dimensional one), and their colour properties as triplets under the colour gauge group $SU(3)_{\text{colour}}$ included by extending $SU(2|1)$ to $SU(5|1)$. Each generation of leptons and quarks (the first being e, ν_e, u, d , the second μ, ν_μ, c, s , the third τ, ν_τ, t and b) can be fitted into the 16-dimensional representation of $SU(5|1)$, which is then reducible to the 15-dimensional representation of $SU(5)$. This latter group has been favoured as the smallest which can contain $SU(2) \times U(1) \times SU(3)_{\text{colour}}$. $SU(5|1)$ may thus be considered as the smallest supergroup which can properly unify the strong, electromagnetic and weak interactions.

As for $SU(5)$ (ref. 7), $SU(5|1)$ also has difficulties over the value of θ_w and proton stability. Latest experimental values of θ_w are nearly incompatible with the known

proton lifetime in $SU(5)$, so that a larger group may be needed. $SU(5|1)$ has also greater latitude in the Higgs boson sector than for $SU(2|1)$, though a neutral Higgs boson mass of twice that of the charged IVB is still predicted, along with numerous other similar particles.

Besides these predictions an interesting feature of the theory is its requirement^{3,6} of extra dimensions whose only evidence is in the Higgs boson structure. Thus the theory is of Kaluza-Klein type. However the extra dimensions are required to be time-like

instead of the traditional space-like ones heretofore. Each new generation of particles requires a further two time-like dimensions for its description; at present there are therefore six extra time-like dimensions alongside our present one and the associated three space-like dimensions. These extra dimensions carry only the symmetry of the theory, but are otherwise trivial. Verification in the 1980s of the predictions of the theory will give support to the existence of these extra dimensions; a very exciting possibility to look forward to.



A hundred years ago

Radiant Matter proceeds in straight Lines

The radiant matter whose impact on the glass causes an evolution of light, absolutely refuses to turn a corner. Here is a V-shaped tube (Fig. 6), a pole being at each extremity. The pole at the right side (*a*) being negative, you see that the whole of the right arm is flooded with green light, but at the bottom it stops sharply and will not turn the corner to get into the left side. When I reverse the current and make the left pole negative, the green changes to the left side, always following the negative pole and leaving the positive side with scarcely any luminosity.

In the ordinary phenomena exhibited by vacuum tubes — phenomena with which we are all familiar — it is customary, in order to bring out the striking contrasts of colour, to bend the tubes into very elaborate designs. The luminosity caused by the phosphorescence of the residual gas follows all the convolutions into which skilful glass-blowers can manage to twist the glass. The negative pole being at one and the positive pole at the other, the luminous phenomena seem to depend more on the positive than on the negative at the ordinary exhaustion hitherto used to get the best phenomena of vacuum tubes. But at a very high exhaustion the phenomena noticed in ordinary vacuum tubes when the induction spark passes through them — an appearance of cloudy luminosity and of stratifications —

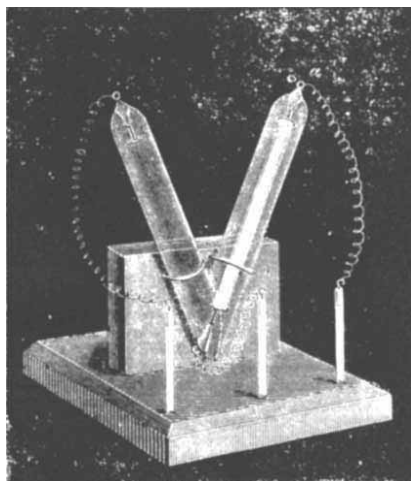


Fig. 6.

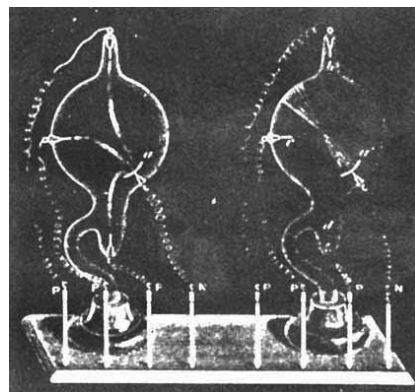


Fig. 7.

disappear entirely. No cloud or fog whatever is seen in the body of the tube, and with such a vacuum as I am working with in these experiments, the only light observed is that from the phosphorescent surface of the glass. I have here two bulbs (Fig. 7), alike in shape and position of poles, the only difference being that one is at an exhaustion as will give the ordinary luminous phenomena — whilst the other is exhausted to about the millionth of an atmosphere. I will first connect the moderately exhausted bulb (A) with the induction-coil, and retaining the pole at one side (*a*) always negative, I will put the positive wire successively to the other poles with which the bulb is furnished. You see that as I change the position of the positive pole, the line of violet light joining the two poles changes, the electric current always choosing the shortest path between the two poles, and moving about the bulb as I alter the position of the wires.

This, then, is the kind of phenomenon we get in ordinary exhaustions. I will now try the same experiment with a bulb (B) that is very highly exhausted, and as before, will make the side pole (*a*) the negative, the top pole (*b*) being positive. Notice how widely different is the appearance from that shown by the last bulb. The negative pole is in the form of a shallow cup. The molecular rays from the cup cross in the centre of the bulb, and thence diverging fall on the opposite side and produce a circular patch of green phosphorescent light. As I turn the bulb round you will be able to see the green patch on the glass. Now observe, I remove the positive wire from the top, and connect it with the side pole (*c*). The green patch from the divergent negative focus is there still. I now make the lowest pole (*d*) positive, and the green patch remains where it was at first, unchanged in position or intensity.

From a Lecture delivered to the British Association for the Advancement of Science, at Sheffield, Friday August 22, 1879 by William Crookes F.R.S.
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review article

Radioisotope clocks in archaeology

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Any absolute dating method requires some form of clock. This review considers those methods in archaeology in which the clock is the rate of disintegration of a radioactive nucleus.

UNTIL about 30 years ago, absolute dating in archaeology was restricted to the interpretation of surviving king lists and the like, while most dating was estimated from stratigraphic or cultural sequences, and their relationship to geological processes. The incorporation of radiocarbon dating has caused a sometimes dramatic modification of accepted ideas, but is now regarded as an essential foundation to archaeological interpretation. Since the discovery of radiocarbon, other dating methods, with application in different circumstances, have been developed; and in the past two years new methods of detecting trace quantities of rare isotopes promise to extend the range of possibilities.

For a given isotope, its rate of disintegration, with its exponential decay law, is essentially completely independent of environmental influences. In this respect it contrasts strongly with the use, for example, of chemical clocks (such as the rate of racemisation of amino acids in degrading bone collagen, or the rate of weathering of flint). However, environment is not entirely without effect. Conditions at the 'start' of the clock must often be inferred, while, of course, there must be a connection between the 'start' and the event being dated. In fact the main challenge of dating lies in the precise articulation of these features. The difficulties of particular methods are considered below, but the example of radiocarbon illustrates the point. For radiocarbon dating, the 'start' of the clock occurs at the death of the organism which is destined to become the sample to be dated. At death, ^{14}C is no longer exchanged with its environment (that is food and atmospheric CO_2), and so the level of ^{14}C decays from its original, constant value. The original level can only be inferred from a consideration of all the processes forming and diluting ^{14}C in the relevant part of the environment, or alternatively by calibration against known-age samples. Biological fractionation and contamination must also be taken into account. With a figure for the original level, and a measurement of the present day level, the time elapsed for exponential decay is simply given by

$$t = 3.32\lambda \log_{10} \left(\frac{L_0}{L_t} \right)$$

where λ is the half life, L_0 the ^{14}C level inferred at the start, and L_t that measured. For ^{14}C the 'start' is comparatively well defined, although the death of a tree, for example, has to be more carefully formulated to apply to metabolic processes of, say, cellulose in an individual tree ring. Archaeologically, the felling of the tree may be regarded as the event to be dated, which does not necessarily correspond to the same thing at all. Also the possible re-use of a timber from an earlier structure makes the purely archaeological interpretation of a radiocarbon date that much more involved.

Archaeology is primarily concerned with the cultural development of man, but this is so closely bound up with man's biological and ecological development that the dating of the latter aspects is of equal importance. Thus while a substantial fraction of archaeological work is concerned with the past 10,000–15,000 yr, dating techniques extending to beyond 5 Myr are necessary. The accuracy of dating depends on the complexity of the information to be resolved. Rapid cultural development may require a date to within ± 20 yr, which, at, say, 6,000 yr BP, corresponds to $\pm 0.3\%$ in the date. Beyond 30,000 yr, where radiocarbon dating is difficult to apply, sites are sparse and dates relatively few. Beyond 100,000 yr the rate of change is likely to follow the rate of environmental, such as climatic, change. The study of environmental change is not only important in its own right, but is also necessary for the proper evaluation of the dating techniques themselves. For example, the change in global ice volume can be followed from oxygen stable isotope measurements in deep sea cores², the sudden release of 'aged' CO_2 dissolved in retreating glaciers can then cause perturbations of the ambient ^{14}C level in the biosphere.

General approaches

The methods considered here can be grouped into three categories: (1) The decrease in concentration of a radioisotope from a presumed initial level, or the reciprocal build-up of a stable daughter product; (2) where a chain of radioactive decays exists, the degree to which the members of the chain are restored to equilibrium following some external initial perturbation; (3) the integration of some local radioactive process by the sample material. The total can then be compared with the value of the local flux, which is usually due to the presence of radioisotopes and remains approximately constant in time.

Methods (1) and (2) technically depend on the need to make accurate measurements of concentrations of radioisotopes or their daughter products, while (3) depends on exploiting a reliable integrating process. In all these cases assumptions must be made about initial conditions, and sometimes about the subsequent history. Often it is not possible to test such assumptions adequately unless a comparison, using a dating method dependent on quite unrelated assumptions, is available. The overlap between different methods has up to now been rather sporadic (mainly owing to differing time scales or materials). The recent developments in ultra-sensitive detection of specific isotopes³, for example, ^{10}Be , ^{14}C , ^{36}Cl , have given a new impetus to the field, and the availability of new methods, with new time scales, should help considerably to bring about such cross-checking.

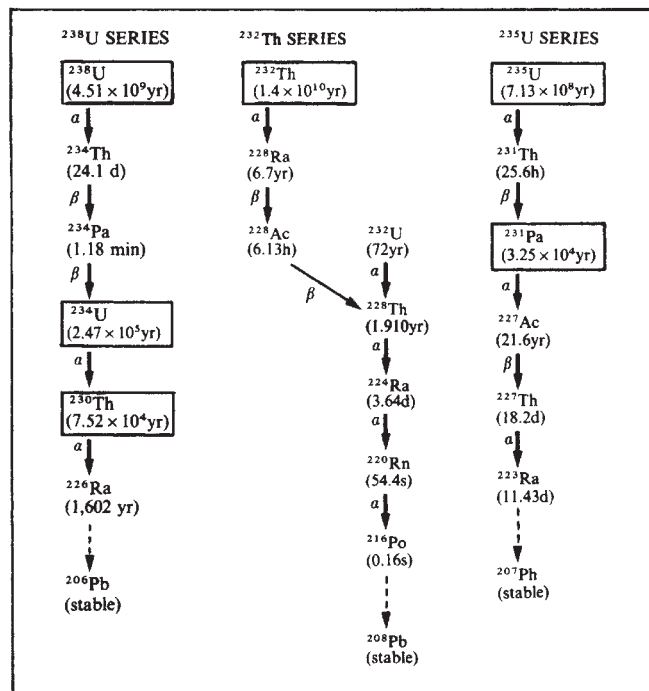


Fig. 1 Uranium and thorium decay series, indicating mode of decay and half life for the nuclides. Long-lived isotopes for dating are enclosed in boxes.

Specific techniques

For convenience we start with the abundant radioisotopes (^{40}K , Th and U), pass to the rare radioisotopes (^{14}C , ^{10}Be , ^{26}Al , ^{32}Si , ^{36}Cl , ^{41}Ca , ^{53}Mn), and finally consider radiation integration techniques (fission track dating, thermoluminescence and related techniques).

K/Ar: ^{40}K occurs with a natural abundance of 0.012% relative to ^{39}K , and a half life of 1.3×10^9 yr. A small fraction decays to ^{40}Ar , so that a measurement of the accumulated ^{40}Ar , together with the quantity of parent ^{40}K (effectively constant over a few Myr) gives the age directly by integrating the exponential decay. This method is primarily applicable to volcanically formed rock such as tuff or pumice. Of course, the rock must not incorporate any additional ^{40}Ar , for example from the atmosphere, and no radiogenic ^{40}Ar must be lost from the rock. Note also that the younger the rock the less radiogenic Ar, and the greater the possibility of contamination and difficulty of measurement. Typically, rocks younger than 50,000–100,000 yr become very hard to date reliably. (The basic method and literature are well covered in ref. 4.)

More recently, the measurement of ^{40}Ar and ^{40}K has been modified in a way which can take account of material in which ^{40}Ar is lost or modified by weathering or reworking. (For reviews, see refs 5 and 6.) In this technique, ^{39}K (which is in constant ratio to ^{40}K) is measured by converting it partially to ^{39}Ar by subjecting the sample to neutron activation. The ^{39}Ar so formed is in a similar micro-environment to that of the entrapped radiogenic ^{40}Ar . On heating the activated sample in incremental steps the ratio of $^{39}\text{Ar}/^{40}\text{Ar}$ can be measured mass spectrometrically, and its degree of constancy indicates the integrity of the material. This is particularly important for the younger samples relevant to archaeology. Measurement of ^{36}Ar allows the proportion of atmospheric Ar contamination to be estimated, and correction for Ca-derived Ar (in the neutron activation) must also be made, especially for young samples.

The necessary assumptions in K/Ar dating are concerned with the particular samples rather than with initial conditions. The $^{39}\text{Ar}/^{40}\text{Ar}$ method allows the validity of these assumptions to be tested for a given case, but real confidence, especially for more recent material, comes from independent dating. Very

satisfactory agreement between K/Ar and fission track dating on the age of the KBS tuff from Lake Rudolph^{7,8} has been obtained. The K/Ar method has been most useful in archaeology in dating the stratigraphy for the finds of hominids. Since it is the rock that is being dated, a stratigraphic relationship must be used to provide the archaeological date.

U: There are several somewhat different methods of uranium series dating. (A general review has been given by Ku⁹.) In the decay of ^{232}Th , or $^{235,238}\text{U}$, a series of daughter products is encountered with a wide range of half-lives. In a closed system each daughter would be in 'secular' equilibrium, but because the geochemistry of some of the longer-lived daughters differs greatly a chemical separation in the environment takes place. The degree of restoration of equilibrium, whose rate is governed by the various half lives involved, can be measured. For example, in the decay of ^{238}U , the first long half life (see Fig. 1) of a non-U member is ^{230}Th ($\lambda = 7.5 \times 10^4$ yr), and this isotope is rapidly precipitated from an aqueous environment. The parent ^{238}U will not then be in equilibrium with its daughters in natural waters. If the uranium is now co-precipitated with, say, calcium as calcite, there will be a build-up of ^{230}Th from a null concentration from ^{234}U and ^{238}U , at a rate in accordance with their half lives, until secular equilibrium is regained. Traces of ^{230}Th from detrital sources which might be incorporated can be estimated, to first order, from a measurement of the ^{232}Th present, if this is small. Often such detritus can be chemically distinguished from the calcite deposit. Provided the initial concentration of ^{230}Th is small, a measurement of the ^{230}Th component of the calcite, together with that of $^{234,238}\text{U}$, allows the age of the calcite since it was formed to be computed. For suitable uranium concentrations this method is applicable to ages in the 50,000–250,000-yr range.

A major application is to carbonates, where the assumptions required are a specification of the isotopic composition during formation of the carbonate precipitate, and the maintenance of a closed system afterwards while equilibrium is being restored. In most cases, it is the isolation of the system that causes more problems. Thus corals seem to be satisfactory subjects¹⁰ while fossil shells do not¹¹. There has been increasing interest in the dating of speleothems (cave deposits), an application with a more direct contact with archaeological interests. Precipitated calcite in these environments seems to constitute a satisfactory closed system, and difficulties over the initial isotopic composition (such as ^{230}Th from dust) are not usually serious. The archaeological applications of speleothem dating by the uranium series method will be reviewed elsewhere¹². Schwartz *et al.*¹³ have previously described an application to a specific archaeological site.

Attempts have been made to extend the method to date bone¹⁴, which strongly accumulates uranium by ion exchange during burial. However, the difficulty of taking into quantitative account the extent to which bone is an open system renders this approach hazardous, and it has not been generally pursued, despite promising results. As one of the extremely few methods that deals with the original archaeological material on a time scale beyond radiocarbon, this application deserves more attention.

^{210}Pb : Although a member of the uranium series, the 22-yr half life of ^{210}Pb puts it into a different sphere of application. Disequilibrium for ^{210}Pb arises from two causes; in terrestrial environments escape to the atmosphere of ^{222}Rn in the decay of ^{238}U brings about a precipitation of unsupported ^{210}Pb as its daughter, and the decay of the ^{210}Pb which finds its way, for example, into lake sediments enables recent stratigraphy¹⁵ to be dated. Archaeological applications to dating using a 200-yr time scale are limited, but one use is in the determination of authenticity. Here disequilibrium is produced in a lead containing artefact during the lead extraction process, so that initially ^{210}Pb is in excess, unsupported by ^{226}Ra . This method has been applied to the dating of lead pigments¹⁶.

The isotopes so far discussed have a terrestrial origin, with parents of very long half lives. The isotopes next to be consid-

ered have a cosmic origin¹⁷, and are formed in minute amounts. For example, the world inventory of ²⁶Al is estimated at only 1 ton. For these isotopes especially, the use of accelerators for their detection³ promises to bring major changes to our present knowledge, although the isotopes considered here have all been detected by conventional methods with one exception (⁴¹Ca). A brief survey of the new possibilities has been made by Arnold¹⁸.

¹⁴C: A comprehensive review of ¹⁴C dating in archaeology has been given by Aitken¹⁹; the subject is too vast to attempt here, but it is worth indicating the similarities and differences between ¹⁴C and the less-studied isotopes.

First, ¹⁴C has some extremely fortunate properties which make it eminently suitable for archaeological dating. These are: a convenient half life (5,730 yr); the ability to equilibrate with the biosphere and so have a direct interaction with man's activities; and a uniform global distribution arising from its equilibration with dissolved oceanic CO₂. Furthermore, ¹⁴C is present in sufficient abundance to provide adequate samples for its measurement (¹⁴C/¹²C = 10⁻¹² for recent material). ¹⁴C is formed by cosmic rays through the (n, p) reaction on atmospheric ¹⁴N, unlike the other isotopes which are predominantly derived from spallation processes. Again unlike the other isotopes (except ³⁶Cl), it is oxidized to a stable gas molecule, CO₂, which equilibrates with the major carbon reservoir of the Earth, the oceans. The level of ¹⁴C in the atmosphere, which is responsible for the level in vegetation and thence in such archaeological materials as charcoal or bone, is maintained by a great number of interrelated processes²⁰. The formation rate^{21,17} depends strongly on the degree of shielding of galactic cosmic rays both by the Earth's magnetic field and by that of the Sun, both of which vary with time. Because there is no energy threshold for ¹⁴C formation (unlike the other isotopes) the formation rate is also more sensitive to bursts of low-energy protons during solar flares. Equilibration with the ocean (or rather the surface mixing layers) has a fractionating effect, which is temperature dependent, and is therefore influenced by global and local climate. Mixing rates with the deep ocean, which is the main reservoir, are not short compared with the half life, and provide a further source of variation. The imprisonment of dissolved CO₂ in glaciers and ice caps has already been mentioned. The well known calibration of ¹⁴C levels^{19,22-24}, based on an independent dendrochronology of samples, shows that for the past 7,000 yr the atmospheric level has varied by <10%. However, the variation actually found shows a very interesting relationship to known changes in the geomagnetic field²⁵, as well as shorter-term changes related to solar activity²⁶. From the immediate archaeological viewpoint these world-wide variations²⁷, enable calendar dates to be derived from the measured residual ¹⁴C level in a sample. We still need to take account of fractionation in the metabolic processes which incorporate the ¹⁴C in the sample, but this can be done through a measurement of the parallel fractionation in the stable isotope ¹³C/¹²C ratio for the same sample. In some cases a rapid fluctuation in atmospheric ¹⁴C level can lead to ambiguous calendar dates, but this is fortunately rare for the period studied. However, it does increase the range of error for a calibrated date. Where a sequence of accurate ¹⁴C measurements can be made, as in

individual growth rings, a very accurate calendar date may be possible by fitting to the appropriate deviation on the calibration curve ('wiggles matching'). For much earlier times, calibration must await an independent, high resolution, alternative dating. There is some indication that ¹⁴C atmospheric levels have remained fairly constant for the past 30,000 yr²⁸, and some evidence that cosmic ray production rates have not varied greatly (less than a factor of 10% on average over the past 10 Myr^{17,29}).

Measurement of ¹⁴C residual levels has hitherto been made by counting the ¹⁴C β-decay rate. This has necessitated large samples (1–5 g), and has placed a limit of 40,000 yr on the age range (extended to 70,000 yr in some special cases). The accuracy of measurement now possible is ±0.2% corresponding to ±16 yr. The typical archaeological sample is rarely dated to this accuracy, however, mainly because of the degree of chemical and mechanical contamination while buried.

An alternative method of measuring ¹⁴C, by counting ions rather than nuclear disintegrations, enables smaller samples to be used (by a factor of 10⁴). This allows new types of material to be dated; for example, iron artefacts containing dissolved carbon, or single bones containing residual collagen, or individual surviving seeds. Samples can be stringently tested and purified to avoid chemical contamination. Also there is much greater choice available in selecting samples. The very low backgrounds in measurements by ion counting technique enables very much older material, perhaps to 100,000 yr, to be dated. All these methods (Figs 2 and 3) are based on a form of mass spectrometry, and they accelerate the ions up to high energies (>1 MeV AMU⁻¹) in order to take advantage of the nuclear techniques of particle identification. For example, at such energies ions of the same mass, charge and energy, such as ¹⁴C⁴⁺ and ¹⁴N⁴⁺, interact differently with matter, losing energy at different rates, and they can therefore be distinguished. Both cyclotrons³⁰ and tandem electrostatic accelerators³¹⁻³³ have been used (see also ref. 3). The Cyclotron provides a mass spectrometer with a very high rejection ratio; the tandem electrostatic accelerator, by virtue of the charge changing interaction at the stripper, can qualitatively reject many particles (for example, molecular ions). The relative merits of each approach depend on many technical issues, including the particular isotope studied, but those groups actively involved in the development of ¹⁴C dating are all using tandem accelerators. It remains to be seen whether the mass spectrometric method can equal the radioactive counter method in accuracy.

¹⁰Be: The ¹⁰Be isotope, as with ²⁶Al and ³²Si, is produced in the upper atmosphere and circulates as an aerosol. Mixing with the troposphere is unevenly distributed throughout the Earth, and the isotope is rapidly precipitated from the troposphere in rain. There is a strong latitudinal variation, reflecting precipitation and mixing rates, and also, at least for ³²Si, a seasonal variation. The quantities of these isotopes reaching the Earth should respond much more sensitively to rapid fluctuations in the cosmic ray flux, since their residence time in the atmosphere is so much less than ¹⁴C (which equilibrates with the ocean surface). ¹⁰Be has been measured in cores from Antarctic ice^{34,35} and also in deep sea sediments³⁶.

¹⁰Be has a half life of 1.5 Myr, and so would provide a suitable time scale to date Pliocene–Pleistocene events. From the strictly archaeological point of view there seems little prospect of any direct connection between an archaeologically significant event and the 'start' of the Be clock, except through the dating of sediments. However, the accurate calibration of sediment cores allows one to calibrate other important stratigraphic measurements, such as changes in fauna and flora, in ¹⁶O/¹⁸O values, magnetic field reversals and changes in inclination and declination. The dating of ocean cores is still at a very early stage^{38,39} and is partly hampered by the need to study the authigenic Be-containing materials of such cores. This implies careful selection of material, and so limits the sample size. Although the way in which ¹⁰Be is deposited in cores is not well understood, good agreement has been obtained between magnetic reversal

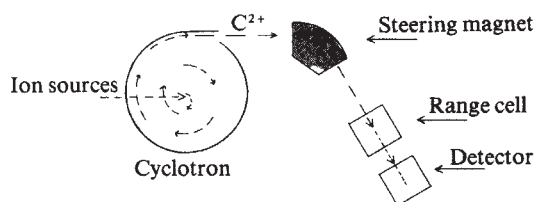


Fig. 2 High-energy mass spectrometry for the detection of ¹⁴C using a cyclotron. The ion source may be external or internal. N²⁺ is removed at the range cell. The detector measures the energy loss rate and total energy of each particle.

dates and ^{10}Be ages measured on the assumption of a constant formation rate, but with a sedimentation rate adjusted to produce a consistent variation of date with depth³⁹.

As for ^{14}C , high-energy mass spectrometry is a suitable technique to apply to ^{10}Be measurement, thus overcoming the main difficulty of the quantity of sample required. By this technique ^{10}Be has been measured in natural samples. In particular, Raisbeck has used a cyclotron to measure ^{10}Be in Antarctic ice cores³⁵, and more recently, in the ocean surface water³⁷. With a measured average of about 700 atoms ml^{-1} , even the atom counting method required the sampling of several cubic meters of water. With the availability of this technique the study of ^{10}Be geochemistry should now develop rapidly. The total residence time of ^{10}Be in the oceans before sedimentation, is particularly important, since this will determine the frequency response to its formation rate of the sedimentary record. For example, the data of Tanaka³⁹, on two cores, suggest that long-term variations (0.7 Myr time scale) are $< \pm 10\%$, and, on a shorter term (0.1 Myr) are $< \pm 40\%$.

^{26}Al : In most respects the situation for ^{26}Al resembles that for ^{10}Be . Its formation rate by spallation is lower, but, contrary to earlier views, the contribution of cosmic dust is apparently not significant⁴⁰. Since any variation in the production of ^{26}Al should parallel that for ^{10}Be , and the two isotopes should have a closely similar geochemistry but different half lives (^{26}Al is 7.3×10^5 yr), it has been suggested that the decay of their ratio provides a time scale less dependent on cosmic ray fluctuations. As with ^{10}Be , ^{26}Al measurements are likely to be of direct importance mainly in the dating of sediment cores. Indirectly the information gained on cosmic ray fluctuations will help to calibrate radiocarbon back to earlier times.

The very low abundance of ^{26}Al ($^{26}\text{Al}/^{27}\text{Al} \sim 10^{-14}$ and below) and its long half life make determination by counting very difficult. ^{26}Al has been measured in ocean sediments by such means⁴¹, and its measurement still poses a challenge to high-energy mass-spectrometric methods. A major problem is the interference from the isobar present in great excess, ^{26}Mg . One way to avoid this is to strip the ions fully, and Raisbeck⁴² has used a linear accelerator as an injector to a cyclotron to produce and separate $^{26}\text{Al}^{13+}$ from $^{26}\text{Mg}^{12+}$. Raisbeck's samples were artificially produced ^{26}Al in the region of $^{26}\text{Al}/\text{Al}$ 10^{-8} – 10^{-11} .

^{32}Si : ^{32}Si has a half life estimated between 250 and 700 yr. It is therefore rather short for archaeological applications, unless it can be applied in contexts beyond the reach of radiocarbon. However, ^{32}Si profiles have been measured in siliceous sediments in the ocean^{43,44} (with the aim of determining the sedimentation rate), and attempts have been made to use the isotope for dating ground waters⁴⁵. However, ^{32}Si in the rain is diluted by the abundant Si isotope as the surface water equilibrates with the local geology. The solubility of Si is determined by many locally varying factors, so that application of the method is very restricted. It is difficult to conceive of any direct archaeological interest, although, of course, an understanding of the local hydrological history of a site might prove important. A clearer

application could be to authenticity measurements, ^{32}Si has not so far been reported as being detected by high-energy mass spectrometry, but is obviously suitable for such an approach.

^{36}Cl : With a half life of 0.31 Myr, ^{36}Cl would seem to have an ideal time scale for extending the archaeological dating range beyond radiocarbon. There is some resemblance to ^{14}C in that the nuclide is likely to have a fairly long residence time in the atmosphere (atmospheric sinks and sources for Cl, which although extensively studied recently, are not known quantitatively), and probably can equilibrate with the surface ocean Cl^- . Furthermore, a considerable proportion of ^{36}Cl may derive, not from the spallation from ^{40}Ar by cosmic rays, but from thermal neutrons by the process $^{35}\text{Cl}(n, \gamma)^{36}\text{Cl}$. This could be important in Cl-bearing terrestrial rocks and at the ocean surface. Very few measurements of terrestrial ^{36}Cl have been made (although measurements in meteorites are relatively plentiful), and these are made more difficult by the lack of positive identification of ^{36}Cl from the weak β radiation counted. One such early study of ^{36}Cl in rain⁴⁶ concluded that considerable enhancement from bomb-testing had taken place. Note that this work required a 2,000 gallon sample. The Rochester/Toronto group⁴⁷, with a tandem accelerator, used about 1–5 l of water and achieved a background sensitivity of $^{36}\text{Cl}/\text{Cl} \sim 10^{-15}$. As with ^{26}Al and ^{41}Ca , the presence of an isobar (^{36}S) in great excess is troublesome. In this case chemical methods (zone-refining) were used to reduce the ^{36}S level to a value where, although the dominant detected ion was at mass 36, the detector was no longer overwhelmed by the ^{36}S count rate.

Two types of application seem possible for ^{36}Cl (ref. 48). The concentration of ^{36}Cl in terrestrial rocks remains to be positively determined, but since 1–2 m of rock would be a sufficient shield to prevent ^{36}Cl build-up it might prove possible to date the time of exposure of a new face, or possibly the burial of formerly exposed material. For example, events such as the emplacement of a glacial erratic, burial by volcanic action, or the occurrence of earthquakes could be studied. This would be a significant extension to the range of terrestrial dating methods for the Pleistocene period. The second type of application is concerned with the solution geochemistry of Cl. The environmental measurements made so far have all concentrated on this aspect, but for quantitative work the major question is to determine the extent to which the groundwater system is closed. Again, as with ^{32}Si , an understanding of the former hydrological status of a region may be vital information in reconstructing its ecology. As for dating associated material, contemporary sediments may possibly incorporate sufficient Cl for dating purposes. Thus argillaceous sediments, possibly speleothems, as well as evaporites might be useful.

^{41}Ca : The isotope ^{41}Ca , with a half life of 0.13 Myr, is more probably formed by $^{40}\text{Ca}(n, \gamma)^{41}\text{Ca}$ than as spallation products in the upper atmosphere. Thus in many ways it resembles ^{36}Cl , and it has been suggested that it could be useful for dating in the same way⁴⁹. Ca participates much more actively in geochemical cycles, and especially as a major constituent of bone, and so would seem to be richer in possibilities.

The equilibrium ratio $^{41}\text{Ca}/^{40}\text{Ca}$ at the Earth's surface would be expected to be in the region of 10^{-14} , which should not be beyond the range of high-energy mass spectrometry. However, the presence of ^{41}K as an isobar is an obvious problem. It could be possibly differentiated from ^{41}Ca by total stripping, as with ^{26}Al , but the energy and therefore the accelerator to accomplish this would have to be very large (>300 MeV), although not beyond present-day possibility.

As with ^{36}Cl , ^{41}Ca could be used to date the exposure of formerly buried rock. (The shielding depth is estimated to be about a metre.) Alternatively, the date of burial of surface material whose ^{41}Ca was in equilibrium could be studied. Since Ca is relatively abundant, its range of applications could be quite large. Again as with Cl, the dating of material formed from Ca-bearing solutions can also be attempted. The usefulness of this method will depend on how reliably the ^{41}Ca content of the relevant hydrological system can be estimated; for example, the

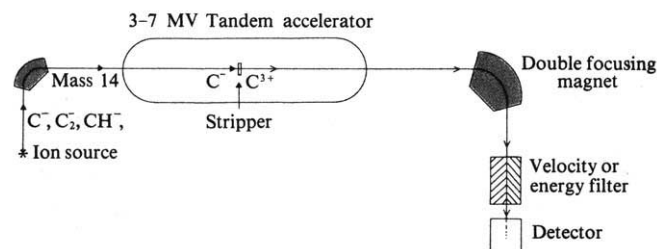


Fig. 3 High-energy mass spectrometry for the detection of ^{14}C using a tandem accelerator. Only ions of mass 14 are injected into the accelerator. Accelerated positive ions are further selected according to their magnetic rigidity and velocity or energy, before measurement of their energy loss rate and total energy in the detector.

expected level of ^{41}Ca deposited in growing bone. If a bone of known initial ^{41}Ca was rapidly buried, or left in a cave, the subsequent decay of ^{41}Ca (provided there was no further exchange) would enable it to be dated just like radiocarbon. The same would apply to the formation of speleothems, with the advantage of a cross check. Such possibilities make ^{41}Ca one of the most interesting cosmogenic radionuclides for archaeological dating, but also technically the hardest to detect. Also, as there is no equilibration with large reservoirs, local fluctuations of ^{41}Ca concentrations may render it too inaccurate to be useful.

Heavier isotopes

Heavier isotopes are also suitable candidates for detection by high-energy mass spectrometry. Their presence on Earth is due to factors such as the infall of cosmic dust, and micro-meteorites. One application, conceivably relevant to archaeology, dated the age of a meteorite fall at 4 Myr from measurements of its ^{53}Mn and ^{36}Cl concentrations⁵⁰. Ocean sediments and volcanic deposits are likely to be the main subjects for study for ^{53}Mn (half-life 3.7 Myr) and ^{129}I (half life 17 Myr). Other isotopes could be included¹⁸ and when their quantitative study is possible, further and unexpected details about the history of the Earth will emerge. For example, knowledge of the overall cosmic ray flux could help with ideas on the extinction of the dinosaurs.

Fission track dating

In this method⁵¹ the accumulation of the fission events of ^{238}U in a material is measured, in contrast to the measurement of the quantity of atoms undergoing radioactive transformations. Many materials, especially glass (for example, obsidian, man made), minerals such as zircons or micas, and rock types such as tuff, basalt or granite, reveal, after etching, microscopic tracks recording each fission event. The density of tracks within a sample increases linearly with time (for archaeological time scales) and the absolute age since the formation of the sample can be derived simply by exposure to a calibrated neutron flux.

The practical application of the method depends on the availability of suitable materials, which must contain sufficient uranium, or be of great enough age, to have accumulated a measurable density of tracks. The dating of the KBS tuff already mentioned is a good example⁸. Other examples are to be found in the review by Fleischer⁵², although such geological applications are not as common as might be expected. As a rule, man-made materials are too recent or contain too little uranium for useful dating by this method, although occasional exceptions, such as a high uranium glaze, are known. A more useful application is where geological tracks in a natural material have been annealed by heat, perhaps accidentally, in a man-made fire, and the time of resetting of the 'clock' can then be measured. For example, an obsidian spearhead approximately 5,000 yr old was 'dated' in this way⁵³.

Thermoluminescence

The well-established use of thermoluminescent dosimeters serves to demonstrate the ability of some materials to integrate α , β and γ doses as low as those occurring in the natural radiation environment of, for example, pottery. Several components of pottery itself have been found to give sufficient thermoluminescence (TL) when integrated over archaeological times. The firing of a pot erases the accumulated geological TL, and subsequent build-up of TL proceeds approximately linearly with time during the burial of a sherd, until the TL is 'read-out' by the rapid heating of a small sample (a few milligrammes, see Fig. 4). Electrons, produced as a result of the radiation dose, remain trapped in the lattice until a level of thermal energy is reached enabling them to recombine. Light produced by recombination near a luminescent centre is detected with a photomultiplier. The sensitivity of the particular sample to radiation is separately

calibrated with a laboratory source. (The application to archaeological dating is reviewed in ref. 54.)

Various difficulties beset the technique and limit the accuracy that can be expected from it. Since TL relates directly to archaeological material it is potentially of great value, and much effort has gone into reducing the complications. First, the radiation dose is hard to assess accurately; the dose from surrounding burial soil is a significant contribution, and although measurable on site, is affected by burial soil moisture; the water content of the potsherd must be considered; and the likelihood of disequilibrium in the uranium and thorium daughters present in the fired clay is another difficulty. Some materials, such as feldspars, are known to lose their TL with time, although this does not seem to be true of the most commonly used mineral, quartz. However, the TL response of quartz is somewhat dependent on its thermal and chemical history. It is possible to select for different components of the α , β and γ doses (which arise from internal ^{40}K , U and Th, and daughters, as well as environmental and cosmic radiation) by using grains of differing sizes^{55,56}. Zircons have been used because only the internal U content need be considered⁵⁷. Unfortunately, there is a spatial mismatch between the zoning of U concentration and the regions of high TL sensitivity in a given grain and this makes the analysis exceedingly difficult.

However, despite these drawbacks, the TL method has provided several pottery dates to an accuracy of $\pm 5\%$ and many at $\pm 10\%$, and is useful where radiocarbon dates cannot be obtained. For old (pre-ceramic) sites, where radiocarbon dates suffer from potential contamination by modern ^{14}C , TL has been applied to such materials as hearths⁵⁸ and burnt flint⁵⁹. There are further prospects that the method can be developed for dating lava flows, ocean sediments, and speleothems.

Summary and prospects

Clearly the ^{14}C isotope will continue to be the mainstay of archaeological dating, and is strengthened by its new-found ability to date samples up to 10,000 times smaller than before. The direct interaction of the ^{14}C cycle with archaeological events is an advantage hardly shared by the other cosmogenic nuclides. Furthermore, for dating into the distant past where much less can be assumed about environmental conditions, errors in estimates for initial values of the radionuclide at the 'start' of the clock will be more serious than is usual from radiocarbon dating. For radiocarbon an error of a factor of 2 corresponds to one half life (5,730 yr), not a disastrous error for a time scale at 30,000–100,000 yr. However, for, say ^{41}Ca , a factor of 2 variation in initial level would produce an error of 130,000 yr; the absolute error is greater for other isotopes.

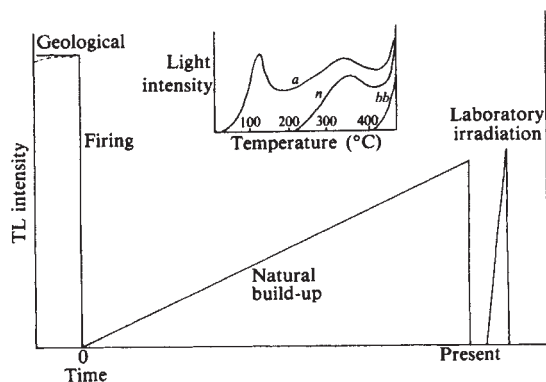


Fig. 4 The build-up of the thermoluminescent signal in pottery with time. Firing the clay erases the geological TL. In the laboratory the accumulated natural TL is measured, and then the TL from an artificial irradiation. Inset shows Glow-curves from a sample: *a*, from artificial irradiation; *n*, from naturally acquired TL over a long period of time; *bb*, the component of light due to black body radiation.

Apart from thermoluminescence, and the possible dating of bone from ^{41}Ca or by U series disequilibrium, most of the dating methods described provide direct dating only through the dating of stratigraphically associated material. This can sometimes be directly impressed by archaeological events as demonstrated by the record of hominid footprints in the Laetoli tuff of Tanzania⁶⁰, material which has now been dated by K/Ar. Note how much archaeological interpretation particularly for the Pleistocene gains from greater knowledge of the environmental changes that have taken place. Clearly ^{10}Be , ^{26}Al , ^{36}Cl in particular can provide a great deal of such information—not necessarily by dating, but by including calibration of the variation in cosmic ray flux, mixing rates for different epochs between

the various terrestrial reservoirs, calibration of geomagnetic changes, and the determination of former local hydrological patterns. Much of this is related to a clearer understanding of the climatic system. Such an ambitious and complex programme is only possible if based on a great quantity of environmental measurements, which has now become possible with the application of high-energy mass spectrometry. The next decade will bring greatly increased quantitative knowledge to the study of climate and its relationship to the development of man.

There is, nevertheless, a gap in archaeological dating beyond the time scale of radiocarbon. At this age man has left few traces; bone, worked tools, fires and disturbed levels are all that we have to 'start' the radioisotope clock.

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articles

Interstellar scintillation and compact sources in supernova remnants

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Interstellar scintillation was detected from a source in or near the supernova remnant G78.1+1.8, implying an angular size $\leq 10^{-6}$ arc s, a brightness temperature $> 10^{18}$ K, and a distance of ~ 3 kpc. The source does not seem to be a pulsar and is therefore the first source other than a pulsar to show interstellar scintillation. We place limits on the scintillating flux for 15 other galactic and extragalactic objects.

ALTHOUGH it is clear that some supernovae produce pulsars, it is by no means certain that all supernovae leave behind compact stellar objects nor that all such objects are pulsars. Recently¹ a new class of objects associated with supernova remnants (SNRs) has been proposed, these are radio sources with spectra markedly different from those of pulsars. While the prime candidate of this new class is probably an extragalactic source², one member of the class, Circinus X-1, is a galactic object that is either a neutron star or a black hole³. The production by supernovae of compact sources that are not pulsars exacerbates

Table 1 Scintillation measurements on 17 radio sources

Source	Position (1950.0)			δ			ν (MHz)	τ (min)	T_{sys} (K)	$\bar{S}_{\text{ISS}}$ (Jy)	Notes
Pulsar calibrator											
PSR0329+54	03 h	29 min	11 s	54°	24′	37″	850	138	167	0.60±0.2	$\Delta\nu_{\text{ISS}} \approx 1.2 \pm 0.1$ MHz
							410	39	189	0.90±0.4	$\Delta\nu_{\text{ISS}} \approx 35 \pm 10$ kHz
Unscintillating calibrators											
0229+34	02	29	05	34	26	55	850	22	162	≲0.12	
3C123	04	33	55	29	34	14	850	54	225	≲0.15	
3C380	18	28	13	48	42	40	850	103	174	≲0.11	
3C449	22	29	06	39	06	14	850	67	164	≲0.12	
2305+18	23	05	17	18	45	12	850	89	158	≲0.11	
3C468.1	23	48	26	64	24	06	410	34	205	≲0.28	
Program sources											
G119.5+10.0	00	14	50	71	56	24	850	298	166	≲0.07	CTA 1
G127.1+0.5	01	25	08	62	50	59	850	196	168	≲0.10	
G132.4+2.2	02	08	08	62	09	30	850	147	167	≲0.09	HB 3
G34.6−0.5	18	54	06	01	15	00	410	19	540	≲0.50	W 44
G69.0+2.7	19	51	15	32	44	00	410	25	232	≲0.30	W 56, CTB 80
G78.1+1.8	20	20	40	40	02	30	850	54	212	0.32±0.1	$\Delta\nu_{\text{ISS}} \approx 1.1$ MHz
							410	16	477	≲0.34	W 66, DR 4
CL 4	20	48	47	31	16	11	850	62	165	≲0.11	
Cyg X-3	20	30	38	40	47	13	850	39	185	≲0.14	
G93.6−0.2	21	22	44	50	51	12	850	78	170	≲0.10	NRAO 662, CTB 104A
NGC7089	21	30	54	−01	03	00	410	23	175	≲0.35	Globular cluster

the already problematic pulsar birth rate, which is just consistent with the supernova occurrence rate if all supernovae produce pulsars^{4,5}. To detect compact sources in SNRs in general and to investigate the recently proposed class of objects in particular, we searched for interstellar scintillations (ISS) produced by electron density fluctuations in the interstellar medium, which occur if the angular size of a source is in the range 10^{-7} – 10^{-3} arc s. Only pulsars have been found to scintillate in the interstellar medium although high-sensitivity observations have been made of extragalactic objects⁶⁻⁹. Here we report upper limits on the scintillating flux of six extragalactic sources, five SNRs, two of the recently proposed compact sources, and a compact source in a globular cluster. In or near one of the SNRs, G78.1+1.8 (also known as W66 and DR4), a scintillating source was detected at 850 MHz which is galactic and does not seem to be a pulsar.

Interstellar scintillation

Scattering of radio waves in the interstellar medium (ISM) produces a stochastic diffraction pattern at the Earth that is convected through the line of sight, thus causing time variations of the signal. The accompanying frequency structure in the spectrum of a radio source has a characteristic frequency scale that is a strong function of the observing frequency. Adopting a power-law model for the electron-density irregularity spectrum in the ISM^{8,10}, we have

$$\Delta\nu_{\text{ISS}} = 8.8 \text{ MHz} \left(\frac{C_N^2}{6.67 \times 10^{-5} \text{ m}^{-6.67}} \right)^{-1.2} \left(\frac{\nu}{850 \text{ MHz}} \right)^{4.4} \left(\frac{L}{1 \text{ kpc}} \right)^{-2.2} \quad (1)$$

as the decorrelation bandwidth (the half-width at half maximum of the autocorrelation function of the radio spectrum) of the ISS at a radio frequency ν . Here C_N^2 is a scale factor for the electron-density spectrum, estimated from ISS of pulsars, and L is the path length through the Galaxy to the source. The level of

scintillation can be quantified by the scintillation index, m_{ISS} , which is the r.m.s. of the radio spectrum divided by the mean, and is sensitive to the angular size of the source. It can be shown⁸ that

$$m_{\text{ISS}} \approx \phi_c / \phi \quad \text{where} \quad m_{\text{ISS}}^2 \ll 1 \quad (2)$$

where ϕ is the source size and ϕ_c is a critical angular diameter given by⁸

$$\phi_c = 0.5 \times 10^{-6} \text{ arc s} \left(\frac{C_N^2}{6.67 \times 10^{-5} \text{ m}^{-6.67}} \right)^{-0.6} \left(\frac{\nu}{850 \text{ MHz}} \right)^{1.2} \left(\frac{L}{1 \text{ kpc}} \right)^{-1.6} \quad (3)$$

While equations (1) and (3) are based on the observed scintillation properties of pulsars, the ISM is inhomogeneous and, for given sources, the predicted decorrelation bandwidths are uncertain by an order of magnitude or more. The Vela pulsar, for example, has a decorrelation bandwidth a thousand times smaller than the predicted value, largely because of the enhanced level of turbulence in the Vela SNR¹⁰.

Observations

Observations were made in July 1978 at 850 MHz and February 1979 at 410 MHz using the 91-m transit telescope of the National Radio Astronomy Observatory. The initial frequency of 850 MHz was chosen as a compromise between maximising the scintillation index and the flux of two of the more promising sources, G127.1+0.5 and CL4. Our source list (Table 1) also included a pulsar (PSR0329+54); six non-scintillating sources of various flux densities; six SNRs with associated compact sources or with 'filled' shells; the radio source near the centre of the globular cluster NGC7089 (ref. 11); and the variable radio source associated with Cyg X-3. An autocorrelation spectrometer was used to obtain 96-point spectra with 10 MHz and

1.25 MHz total bandwidths for each of two orthogonal linear polarisations. Approximately 10–30 spectra, each with integration time 1 min, were recorded at the source position, followed by an equivalent set of off-source spectra from the same declination and 10–30 min east of the on-source position. Normalised spectra were calculated from

$$S(\nu) = T_{\text{on}}[S_{\text{on}}(\nu) - S_{\text{off}}(\nu)]/S_{\text{off}}(\nu) \quad (4)$$

where T_{on} is the on-source system temperature and S_{on} and S_{off} are the on- and off-source spectra.

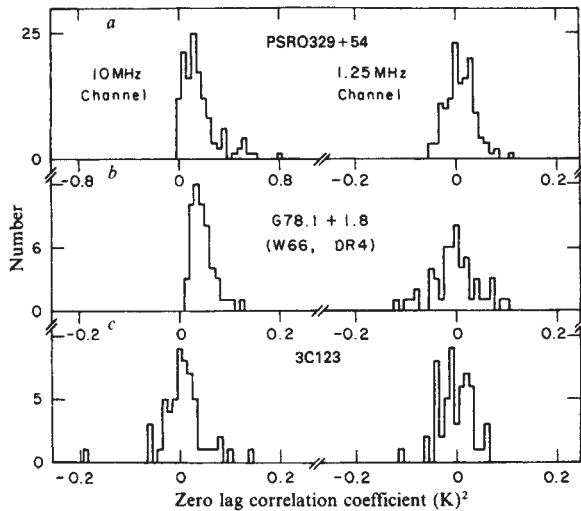


Fig. 1 Histograms of the zero-lag cross-correlation coefficient (see equation (5) with $l=0$) for three radio sources: *a*, PSR0329+54; *b*, G78.1+1.8 (W66, DR4); *c*, 3C123. Interstellar scintillation generates frequency structure that is positively correlated between the spectra of two polarisations. Spectra with total bandwidths of 10 MHz and 1.25 MHz were analysed.

Our technique for searching for ISSs takes advantage of the fact that ISS are correlated between the two polarisations while radiometer noise and, to some extent, quasi-periodic baseline fluctuations due to standing waves are uncorrelated. Letting $S_1(k)$ and $S_2(k)$ be the sampled spectra for the two polarisations, the cross-correlation function (CCF),

$$\rho_{12}(l) = \frac{1}{N-l-1} \sum_{k=0}^{N-l-1} S_1(k)S_2(k+l), \quad l=0, 1, \dots \quad (5)$$

was calculated for each 1-min integration. These were summed to form an average correlation function. Histograms of the zero-lag values were formed and were extremely useful in indicating the extent to which baselines of the spectra have structure that is uncorrelated between the two polarisations. Figure 1 shows these histograms of the zero-lag points of the 1-min correlation functions for three sources. The histograms for 3C123 are symmetric about zero, as expected if radiometer noise and uncorrelated baseline fluctuations are the dominant components of the spectra. The widths of the histograms are approximately $\sigma_p \approx \alpha T_{\text{on}}^2 / \delta\nu \tau N^{1/2}$ if only radiometer noise contributes, where τ is the integration time, N is the number of channels of bandwidth $\delta\nu$, and α is a constant of the order of unity. The histogram for the 1.25 MHz total bandwidth conforms to the radiometer noise value of σ_p , whereas the 10 MHz total bandwidth exceeds the radiometer noise value by a factor of 5. The excess arises from quasi-periodic baseline fluctuations

with ~ 1 cycle every 4 MHz at 850 MHz. Such fluctuations were suppressed by subtracting a low-order least-squares polynomial from each spectrum before cross-correlating. The data presented here had a first-order polynomial subtracted. We find that the quasi-periodic baselines are generally uncorrelated between the two polarisations and that they change in amplitude and phase from one integration to the next.

ISS are evident in Fig. 1 for PSR0329+54 and G78.1+1.8 in the histograms for the 10 MHz spectra. The values of $\rho_{12}(0)$ are definitely positive for these sources, signifying that correlated structure is always present. While the presence of correlated structure is necessary to detect ISS, it is not sufficient to prove that ISS are in fact being observed. However, the shapes of the average CCFs confirm that ISS are the cause of the correlated structure. These are shown in Fig. 2. Of the 17 sources, only two, PSR0329+54 and G78.1+1.8, show correlation functions that peak at zero lag with amplitudes that are significantly larger than the magnitudes of negative excursions of the CCFs. Moreover, the shapes of the CCFs are stable from one day to the next for these sources. The quasi-periodic CCF for 3C123 peaks at a non-zero lag and attains negative values equal in magnitude to the maximum positive values as would be expected for quasi-periodic baseline structure. The CCF for the weaker calibration source 3C380 is consistent in amplitude with estimation errors expected from radiometer noise only, indicating that standing waves are not important for this source.

Note that if Faraday rotation of a linearly polarised source were significant over the total bandwidth, the resultant periodic structure would be anti-correlated between the two polarisations because the spectrum in each is proportional to $\sin^2(\beta\nu)$ and $\cos^2(\beta\nu)$, respectively, where β is related to the rotation measure. Such an effect is not apparent for any of the sources nor does the integrated linear polarisation of PSR0329+54 (which is small at 850 MHz) seem to influence the correlation functions significantly.

Table 1 shows the positions of the sources, the frequency, integration time, and system temperature; for the two sources showing ISS we list the scintillating flux density derived from

$$S_{\text{ISS}} = C[\bar{\rho}_{12}(0)]^{1/2} G^{-1} \quad (6)$$

where $\bar{\rho}_{12}$ is the average correlation function, $C \approx 1.15$ is a correction factor for the flux density lost in the baseline removal process, and $G \approx 0.8 \text{ K Jy}^{-1}$ ($1 \text{ Jy} = 10^{-23} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ Hz}^{-1}$) is the system gain. A three sigma error on the scintillating flux density is

$$3\sigma_{\text{ISS}} \sim 3C\sigma_p^{1/2} / GM^{-1/4} \quad (7)$$

where σ_p is the measured r.m.s. of the set of M zero-lag correlation coefficients for each minute of integration time. Crude estimates of the decorrelation bandwidth, $\Delta\nu_{\text{ISS}}$, were obtained by taking the lag at which the CCF crosses zero. For PSR0329+54, the two decorrelation bandwidths are consistent with values of 1.1 MHz and 43 kHz predicted by equation (1) at 850 MHz and 410 MHz, respectively.

For the remainder of the sources, the upper limit on S_{ISS} given in Table 1 is the larger of the three sigma values given by equation (7) for the 10 MHz and 1.25 MHz total bandwidths. Although ISS were detected at 850 MHz for G78.1+1.8, they were not evident at 410 MHz above the rather high upper limit.

The scintillating source G78.1+1.8

The ISS from G78.1+1.8 cannot be easily associated with any particular source for two reasons. First, the 16 arc min telescope beamwidth at 850 MHz encompasses a complex region containing several radiosources. Second, the nature of the observations (see equation (4)) implies that ISS could have arisen from our off-source position of $\alpha(1950.0) = 20 \text{ h } 34 \text{ min } 00 \text{ s}$ and

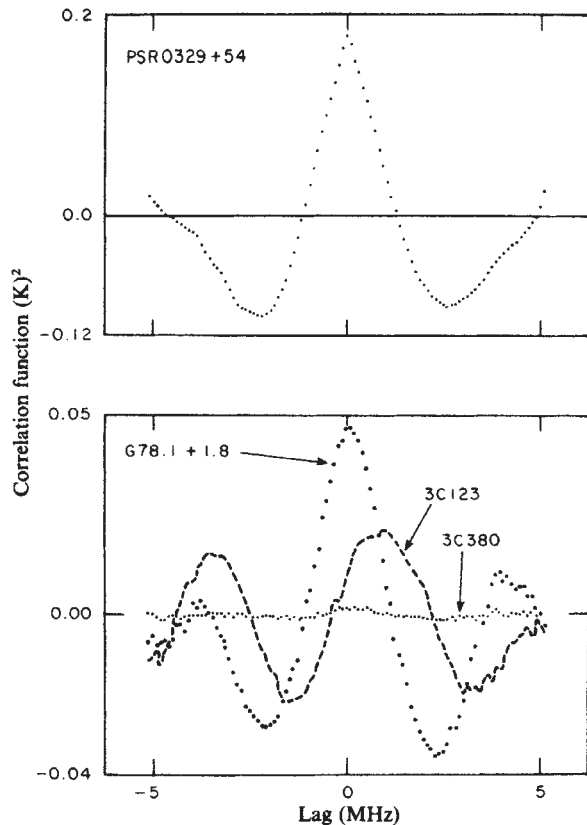


Fig. 2 Average cross-correlation functions for four sources at 850 MHz. Each lag is a multiple of the spectral resolution, 104 kHz.

$\delta(1950.0) = 40^\circ 02' 30''$. It would not be surprising if the source were within the SNR W66, especially since the position of a γ -ray source, CG78+1, has an error box that contains W66 (ref. 12). However, the ISS may be associated with a point source¹³ at $\alpha(1950.0) = 20 \text{ h } 20 \text{ min } 19 \text{ s}$, $\delta(1950.0) = 40^\circ 06' 09''$ which was within our beam. Although this source is variable, our observations require the flux density to have been an order of magnitude larger than the 850 MHz value expected from the measured values of 68 mJy at 1,415 MHz and 10–30 mJy at 610 MHz¹³.

An obvious possibility is that the source is a pulsar. However, observations at 410 MHz using an on-line search program¹⁴ showed no evidence of radio pulses above an average flux density of 20 mJy over a period range from 33 ms to 4 s and with a dispersion measure $< 120 \text{ pc cm}^{-3}$. The on-source and off-source positions have also been covered in two previous pulsar searches⁴ that were sensitive down to at least 80 mJy. Moreover the limit on ISS at 410 MHz suggests a spectrum for the source that declines toward decreasing frequencies (that is unlike a pulsar), although the source variations may have decreased the flux density below our detection threshold during 16-min integration made at 410 MHz.

By using equations (1)–(3), we can place limits on the distance, the angular size, and the brightness temperature of the source. The similarity of the decorrelation bandwidth to that of PSR0329+54 implies a similar distance, $\sim 2.6 \text{ kpc}$, although this is uncertain by at least a factor of two. Assuming this distance, we can relate the source flux density, S , to the observed

scintillating flux density by

$$S = S_{\text{ISS}}/m_{\text{ISS}}$$

as long as $m_{\text{ISS}}^2 \ll 1$. There were no unresolved sources in our beam with $S > S_{\text{max}} = 1.2 \text{ Jy}$ ¹³ and therefore $\phi \leq 10^{-7} (S_{\text{max}}/S_{\text{ISS}}) \text{ arc s}$. Consequently, the brightness temperature,

$$T_B = \lambda^2 S / 2k_B \phi^2 \geq 10^{18} \text{ K}$$

(where k_B is Boltzmann's constant) exceeds by six orders of magnitude the 10^{12} K maximum for incoherent electron-synchrotron radiation from a stationary source, which is limited by Compton scattering of synchrotron photons off the relativistic electrons. Although relativistic blast-wave models¹⁵ with lorentzian factors γ can yield maximum brightness temperatures of 10^{12} K , it is probably not possible to have a source as small (linear size $\leq 20\%$ of a solar radius) as we have inferred without self absorption. Consequently, our results suggest that a coherent radiation mechanism is responsible for the radiation.

We conclude that the source may be a pulsar with a period $< 33 \text{ ms}$ or a pulsar of arbitrary period whose pulses are smeared out by enhanced dispersion from a region near the source. Any residual pulsation must be at a level $\leq 20 \text{ mJy}$ or a short-period pulsar must have a flux density $\geq 0.30 \text{ mJy}$ at 410 MHz. Either case implies an extraordinary flat spectrum as far as pulsars go. If it is not a pulsar, the source may be similar to that associated with Cir X-1, which is a binary system in which high-energy photons, produced by matter accreting on a compact object, drive a shock wave that accelerates electrons to lorentzian factors of 10^3 – 10^5 (ref. 3).

Compact sources in SNR

Other than G78.1+1.8, no compact sources were found in or near any of the six other supernova remnants above a flux density of $\sim 100 \text{ mJy}$ at 850 MHz. For G127.1+0.5 this is not surprising as the central compact source has a VLBI-determined size of $5 \times 10^{-4} \text{ arc s}$ at 10.7 GHz (ref. 16). Three point sources, 4C71.01, 4C62.05, and 4C50.55, near the SNRs G119.5+10.0, G132.4+2.2, and G93.6–0.2, respectively, were not found to scintillate, signifying that such sources are indeed extragalactic or, if galactic, are too large ($\phi \gg 10^{-7} \text{ arc s}$) to scintillate at a detectable level. The SNRs with filled shells (G34.6–0.5, G69.0+2.7) do not show signs of compact sources, although our limits on these are not strong.

The point source in the globular cluster NGC7089 has a flux density of 1.3 Jy at 410 MHz and a steep spectrum ($\propto \nu^{-1.9}$) (ref. 11). Lack of ISS again argues against the source being an exotic compact object. The path length through the galaxy for this source is 1.7 kpc, assuming a disk galaxy with thickness 2 kpc, so ISS should have been detectable in the 10 MHz channel. As for Cyg X-3, the absence of ISS is not surprising because its emission seems to be consistent with an expanding incoherent synchrotron source¹⁷.

Although our source list is too small to place meaningful constraints on the pulsar birth rate, it is clearly of interest to determine the nature of the source in or near G78.1+1.8. If it is a neutron star but not a pulsar, then the possibility exists that a significant fraction of neutron stars never manifest themselves as pulsars, therefore requiring yet more supernovae per unit time to account for the observed pulsars.

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Basalt magma sources during the opening of the North Atlantic

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The mantle that supplied basalts to the North Atlantic Tertiary province at the time of continental breakup was isotopically similar to the present-day sub-oceanic mantle. There is no evidence for undepleted source regions beneath the continent at this time.

THE determination of both $^{143}\text{Nd}/^{144}\text{Nd}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in oceanic and continental basalts¹⁻⁵ has provided a stimulus to the evaluation of the timing and extent of differentiation in their mantle source regions. Volcanic rocks which erupted along mid-oceanic ridges comprise the largest proportion of terrestrial volcanics and may be expected to provide a good estimate of the isotopic characteristics of the sub-oceanic upper mantle. The relatively radiogenic Nd- and unradiogenic Sr-isotope compositions of mid-ocean ridge basalts (MORB) imply that the sub-oceanic upper mantle has been depleted in Rb relative to Sr and Nd relative to Sm.

The chemical character of the subcontinental upper mantle is a subject of considerable speculation. On the basis of thermal and seismic considerations, some authors^{7,8} favour a markedly depleted subcontinental upper mantle. It is envisaged in these models that the subcontinental upper mantle has been depleted in basaltic constituents, producing a lower Fe/Mg ratio relative to the sub-oceanic mantle. Parts of the mantle which have lost a basaltic melt should not only have a lower Fe/Mg ratio but should also be depleted in the incompatible trace elements and have a lower Rb/Sr ratio and a higher Sm/Nd ratio than undepleted mantle. However, the Nd- and Sr-isotope compositions of continental basalts frequently indicate less depleted source regions than those supplying MORB. In some instances the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of continental basalts (which are often higher than the present-day bulk Earth value^{2,4}) have been taken to infer the existence of ancient source regions beneath the continents that have time-integrated Rb/Sr ratios greater than that of the bulk Earth⁹⁻¹¹. Several factors make it difficult to assess the extent to which continental basalts reflect the average properties of the subcontinental mantle. First, they typically sample only a small proportion of the mantle and are generated at unknown depths. Second, it is possible that they have been contaminated by the continental basement through which they have been erupted¹²⁻¹⁴. In some cases, such as the Tertiary basalts of North-west Scotland, basalts contaminated with continental crust may be readily distinguished from uncontaminated basalts by their isotope and trace element systematics. Third, if the subcontinental upper mantle is markedly heterogeneous, with strongly depleted and thus refractory regions interspersed with less depleted regions⁸, melting events may be expected to sample the less depleted areas preferentially and provide a biased impression of the chemical state of the mantle.

The present project is aimed to obtain further information concerning the chemical characteristics and history of those portions of the subcontinental mantle which have undergone partial melting to produce continental basalt. To this end, Nd-

and Sr-isotope compositions have been determined in plateau and flood basalts erupted between 50 and 70 Myr ago during the initial breakup and opening of the North Atlantic. The major occurrences of plateau lavas and intrusive centres associated with rifting of the North Atlantic are shown on a continental reconstruction in Fig. 1. The major period of rifting, volcanism and subsequent continental drift in the North Atlantic was heralded by a large-scale development of plateau lavas and intrusive complexes along the rifted margins of the proto-North Atlantic Ocean. In North-west Scotland igneous activity began

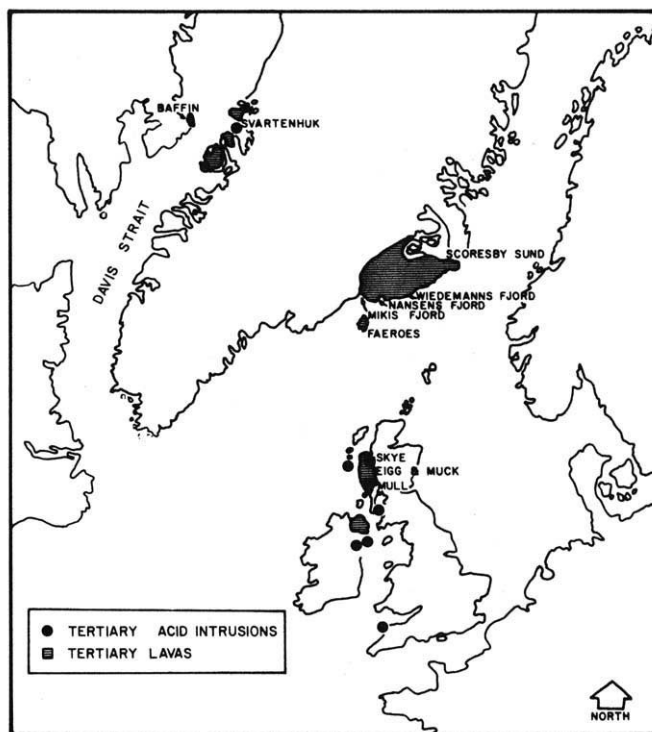


Fig. 1 Location of Tertiary lavas and acid intrusive centres in the North Atlantic region plotted on a continental reconstruction appropriate to ~60 Myr ago.

about 66 Myr ago and continued locally to about 50 Myr ago (refs 15, 16 and R. D. Beckinsale and R. J. Pankhurst, personal communication). The plateau lavas now forming the Faeroe Islands, and occurring in east Greenland and on either side of the Davis Strait in west Greenland and Baffin Island were erupted during the same period¹⁷⁻¹⁹. Of the samples analysed only those from the Faeroes are not definitely known to have been erupted through continental crust; Interpretation of geophysical data for the Faeroes area has been equivocal in this respect^{20,21}. Altogether, the North Atlantic Tertiary volcanic province is comprised of some 2,000 km² of lavas which include mainly

Table 1 Rb-Sr and Sm-Nd data

Sample	Location	Rb (p.p.m.)	Rb/Sr	Sr (p.p.m.)	$^{87}\text{Sr}/^{86}\text{Sr}$	Sm (p.p.m.)	Sm/Nd	Nd (p.p.m.)	$^{143}\text{Nd}/^{144}\text{Nd}$
Faeroes									
Fo 07	Torshavn	1.19	0.004	282	0.70324 ± 3	2.66	0.339	7.84	0.51280 ± 2
Fo 19	Torshavn	9.02	0.07	123	0.70357 ± 5	5.54	0.263	21.1	0.51287 ± 3
Fo 23	Torshavn	2.53	0.009	270	0.70325 ± 4	6.20	0.271	22.9	0.51293 ± 2
Fo 34	Suduroy	11.1	0.041	274	0.70348 ± 5	7.04	0.255	27.6	0.51292 ± 2
Fo 41	Suduroy	1.87	0.005	388	0.70340 ± 4	3.99	0.279	14.3	0.51286 ± 3
Fo 52	Vestmanna	5.89	0.031	188	0.70337 ± 4	4.21	0.277	15.2	0.51298 ± 3
East Greenland									
MM20333	Mikis Fjord	10.7	0.031	349	0.70392 ± 4	5.00	0.285	17.5	0.51288 ± 3
CKB7147	Nansens Fjord	11.0	0.040	277	0.70361 ± 5	6.70	0.255	26.3	0.51277 ± 3
CKB7148	Nansens Fjord	2.53	0.006	362	0.70367 ± 5	6.52	0.261	25.0	0.51280 ± 2
EG 7207	Wiedemans Fjord	4.28	0.017	255	0.70363 ± 5	5.28	0.268	19.7	0.51286 ± 3
EG 7203	Wiedemans Fjord	13.1	0.058	244	0.70336 ± 4	5.59	0.261	21.4	0.51283 ± 2
EG 7126	Scoresbysund	4.35	0.020	214	0.70330 ± 8	5.24	0.296	17.7	0.51288 ± 2
Baffin and Svartenhuk*									
B 18	Cape Dyer	0.87	0.007	128	0.70330 ± 4	2.24	0.299	7.50	0.51290 ± 3
B 22	Cape Dyer	1.96	0.006	314	0.70365 ± 4	2.01	0.294	6.83	0.51287 ± 3
B 36	Cape Dyer	1.78	0.013	131	0.70412 ± 4	2.73	0.320	8.53	0.51277 ± 3
S 8	Svartenhuk	2.56	0.021	121	0.70326 ± 4	2.74	0.302	9.07	0.51299 ± 3
S 16	Svartenhuk	14	0.099	141	0.70302 ± 4	2.34	0.326	7.16	0.51301 ± 3
S 32	Svartenhuk	2.13	0.010	221	0.70374 ± 4	5.37	0.342	15.7	0.51289 ± 3
S 41 (trachyte)	Svartenhuk	62	0.57	108	0.70365 ± 4	10.3	0.200	51.4	0.51287 ± 2

$^{86}\text{Sr}/^{88}\text{Sr}$ normalised to 0.1194; $^{146}\text{Nd}/^{144}\text{Nd}$ normalised to 0.7219; ratios are initial ratios computed for 60 Myr ago. (Eimer and Amend $^{87}\text{Sr}/^{86}\text{Sr} = 0.70800$.)

* Some of the abundance data reported from R.K.O'N. and Clarke⁴.

alkali olivine basalts, but also tholeiites, hawaiites mugearites and trachytes. Central intrusive complexes occur throughout the region but are most strongly concentrated in North-west Scotland (Fig. 1) and may include periodotites, gabbros and granites.

Nd- and Sr-isotopes

$^{87}\text{Sr}/^{86}\text{Sr}$ and $^{143}\text{Nd}/^{144}\text{Nd}$ ratios together with Rb, Sr, Sm and Nd concentrations have been determined in 19 basalt samples from the Faeroe Islands, east Greenland, west Greenland (Svartenhuk) and Baffin Island (Cape Dyer). Some petrological and geochemical features of basalts from these regions have been discussed previously²²⁻²⁴. The isotope ratios presented in Table 1 are computed initial ratios assuming an age of 60 Myr; small uncertainties in the estimated time of eruption result in negligible errors in the quoted initial ratios. Within each of the provinces (Faeroes, east Greenland, Baffin and Svartenhuk) there are significant variations in the initial $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{143}\text{Nd}/^{144}\text{Nd}$ ratios. $^{143}\text{Nd}/^{144}\text{Nd}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ exhibit a strong negative correlation in the case of the Baffin Island and Svartenhuk samples, but only a poor one in the case of east Greenland and Faeroes samples (Fig. 2). If all the samples are considered together, however, there is a good negative correlation between these two parameters, and the range of isotope compositions is similar to that for more recent volcanics erupted in parts of the North Atlantic Ocean basin (Fig. 2)²⁵. More specifically, the Nd- and Sr-isotope compositions overlap with those of recent Mid-Atlantic ridge basalts and possess a similar range to Icelandic basalts erupted over the last 16 Myr (refs 4, 24). The plateau basalts are compared with the bulk Earth Rb/Sr and Sm/Nd ratios using the parameters ΔNd and ΔSr ⁶ in Fig. 3, from which it is evident that they were all derived from source regions previously depleted in Rb relative to Sr and Nd relative to Sm and in this respect are similar to all basalts subsequently erupted in the North Atlantic. The Rb/Sr ratios of these basalts range from 0.004 to 0.099, both less and greater than the model bulk Earth value of 0.03 (refs 2, 4), and Sm/Nd ratios range from 0.255 to 0.342, again less and greater than the bulk Earth value of 0.31 (refs 1, 4). This feature of variable Rb/Sr and Sm/Nd ratios is not uncommon amongst basalts erupted in the ocean basins. There are no clear general correlations between the Sm/Nd, Rb/Sr, $^{143}\text{Nd}/^{144}\text{Nd}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in these basalts.

Localisation of crustal contamination

In addition to the analyses presented here, Nd- and Sr-isotope data are also available for Tertiary basalts from North-west Scotland (Skye, Mull, Eigg and Muck)¹⁴. The major contrast between the North-west Scotland basalts and those from the other locations in the North Atlantic province is the clear divergence of the $^{143}\text{Nd}/^{144}\text{Nd}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of some basalts from the trend defined by the results presented here and for other recent North Atlantic basalts (Fig. 4). These divergences have previously been attributed to contamination of the basalts by the 2.9-Gyr-old Lewisian basement gneisses through which they were erupted. In some instances this contamination involved Lewisian granulite facies material with negative ΔNd

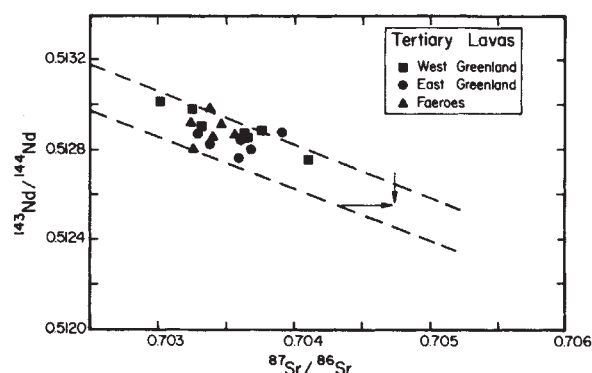


Fig. 2 Comparison of $^{143}\text{Nd}/^{144}\text{Nd}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in North Atlantic volcanics (Table 1) recalculated for their time of eruption 60 Myr ago and compared with the bulk Earth $^{143}\text{Nd}/^{144}\text{Nd}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios (indicated by arrows) and inferred bounds of contemporary Atlantic basalt source regions at that time.

values in the range -30% to -40% and ΔSr values between -20% and -40%. Elsewhere, Lewisian amphibolite facies material with similar ΔNd values, but with positive ΔSr values of more than 100%, seems to have been the contaminant.

So far, there is no evidence for basalt contamination by Precambrian basement elsewhere in the Tertiary North Atlantic.

It is evident that contamination is a common but localised feature of the North-west Scotland basalts and correlates with the concentration of Tertiary acid intrusive centres in this region (Fig. 1). The Pb-, Nd- and Sr-isotope compositions of the acid intrusives in Skye, North-west Scotland^{14,26}, indicate that Lewisian basement is a major component of their source. This may reflect overall higher temperatures in the lower crust in North-west Scotland at the time of basalt eruption than elsewhere in the North Atlantic Tertiary province.

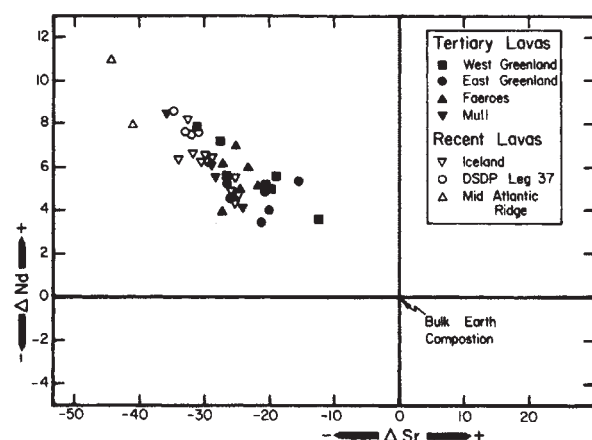


Fig. 3 Comparison of ΔNd and ΔSr values in Tertiary lavas from Baffin Island, east and west Greenland, Faeroes, Mull and Recent volcanics from the North Atlantic⁴. The solid square symbol is used for both Svartenhuk (west Greenland) and Baffin Island samples. ΔNd is the percentage deviation of the time-integrated Sm/Nd calculated from the measured $^{143}Nd/^{144}Nd$ ratio from the bulk Earth value, and ΔSr is the analogous parameter for Rb/Sr ratios⁶.

Evolution of the North Atlantic source regions

The Nd- and Sr-isotope compositions of the basalts reported here and those of uncontaminated basalts from Northwest Scotland¹⁴ all indicate derivation from source regions previously depleted in Rb relative to Sr and Nd to Sm. This observation is reflected in their uniformly positive ΔNd and negative ΔSr values. There is no evidence for the involvement of undepleted (or pristine) mantle source regions in the generation of basalts during the breakup of the North Atlantic, or indeed any basalt samples with either positive ΔSr or negative ΔNd values which are not readily explained by crustal contamination. The isotopic evolution of the subcontinental mantle sources sampled in the Tertiary seems to have been similar to that of some of the sub-oceanic sources sampled subsequently during the generation of the North Atlantic Ocean basin and particularly those represented by Icelandic volcanics erupted during more recent times (Fig. 3).

It is interesting to compare these conclusions with inferences concerning the chemical characteristics of subcontinental mantle based on geophysical observation and other continental volcanic suites. Both Jordan⁷ and Oxburgh and Parmentier⁸ have suggested that the subcontinental mantle must be overall more depleted in basaltic constituents than suboceanic mantle in order to stabilise it against convective disruption. If the degree of depletion of subcontinental mantle were uniform and had occurred over the same time scale as the depletion of present-day sub-oceanic mantle, continental basalts would have extreme values of ΔNd and ΔSr , similar to those of MORB. However, the isotopic characteristics of Tertiary North Atlantic basalts are more similar to those of some contemporary Icelandic basalts. If the Tertiary basalts were derived from the less depleted parts of the subcontinental mantle and other more-depleted and refractory areas did not melt, the basalts may convey a biased impression of the degree of depletion. Alternatively, and perhaps more likely, any depleted subcontinental mantle existing beneath the region before breakup may have been disrupted and replaced by less-depleted mantle material at a higher temperature and with a geological history similar to that of mantle material subsequently sampled during the evolution of the North Atlantic Ocean basin.

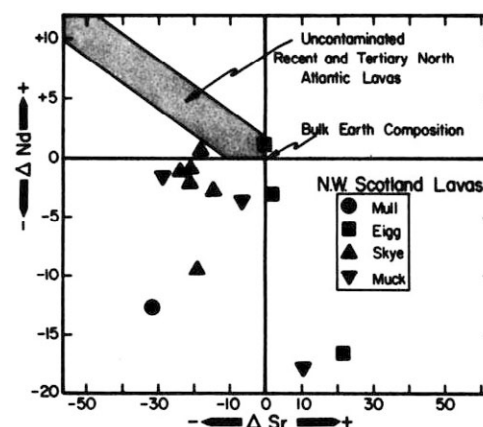


Fig. 4 Comparison of ΔNd and ΔSr parameters for Tertiary and Recent uncontaminated volcanics from the North Atlantic with Tertiary North-west Scotland volcanics inferred to have been contaminated by Lewisian basement⁵. ●, Mull; ■, Eigg; ▲, Skye; ▼, Muck.

Although the data obtained for the Tertiary North Atlantic basalts do not refute claims for the existence of undepleted or enriched subcontinental mantle source regions^{2,9}, they do clearly demonstrate that such potentially fertile regions were not available to produce basalts beneath the North Atlantic region at the time of incipient breakup in the early Tertiary.

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A role for brown adipose tissue in diet-induced thermogenesis

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Measurement of energy balance during voluntary overeating in rats unequivocally establishes the quantitative importance of diet-induced thermogenesis in energy balance. Like cold-induced thermogenesis, this form of heat production involves changes in the activity of the sympathetic nervous system and brown adipose tissue which suggest that this tissue may determine metabolic efficiency and resistance to obesity.

It is generally accepted that heat production in mammals varies, but there is considerable controversy about the factors which cause this variation. Cold-adapted animals show non-shivering thermogenesis (NST) in which increased heat production, not associated with muscular activity, is mediated by the sympathetic nervous system and the animals show a pronounced increase in sensitivity to the thermogenic and lipolytic effects of catecholamines^{1,2}. The capacity for NST is related to the presence of brown adipose tissue (BAT) which is found in many cold-adapted animals, hibernators and the neonates of many species, including man³. *In vivo* measurements indicate that, in spite of its small total mass (1–2% of body weight in the cold-adapted rat), under the influence of catecholamines BAT can receive as much as 34% of cardiac output and probably accounts for up to 60% of NST in the cold-adapted rat⁴. The significance of diet-induced thermogenesis (DIT) remains controversial. It is generally agreed that genetic obesity in mice is, in part at least, due to a failure of DIT^{5,6} (associated also with a failure in NST^{6,7}). There is also considerable evidence to show that variation in the energy cost of weight maintenance or weight gain can be induced in rats^{8–11}, pigs^{8,12} and man^{13–15} by manipulating dietary composition or intake. Miller¹⁶ has argued that DIT has a central role in the regulation of energy balance, whereas Blaxter¹⁷ has suggested that DIT is relatively unimportant. The controversy arises mainly from the use of abnormal animals and diets to produce differences in energy intake, in contrast to the voluntary overfeeding used in human studies. We have now developed a new dietary regimen that induces voluntary overeating of a balanced diet in normal animals and unequivocally demonstrates the quantitative importance of DIT in energy metabolism. As with NST, there is a sympathetic/catecholamine involvement and changes in the size and metabolism of BAT suggest that this tissue is an effector of DIT as well as NST.

'Cafeteria' feeding

We have used a dietary regimen originally described by Scalfani and Springer¹⁸ which we call 'cafeteria' diet. Rats are offered various palatable food items in addition to normal laboratory stock diet. Each day four new food items are offered and the rat, which normally controls its intake precisely, is induced to overeat. On this diet animals rapidly become obese but when

palatable food items are removed, and only stock diet is available, body weight rapidly returns to the level of animals fed stock diet throughout¹⁹. This reversible obesity seems to have no lasting effects on metabolism: three repetitive bouts of treatment do not affect subsequent ability to lose weight²⁰.

The degree of obesity obtained using the cafeteria diet varies and initially this was ascribed to a failure in some experiments to induce hyperphagia. However in all experiments where food intake was measured there was evidence for marked hyperphagia, suggesting that differences in weight gain were due to variations in heat production.

Adult male Sprague-Dawley rats (from Charles River) were allowed free access to water and a pelleted stock diet. Half of the animals were maintained on the cafeteria diet for 21 days after which all animals continued on stock diet only. Detailed measurements of energy balance were made on six animals from each group and the results are shown in Table 1. In spite of consuming 80% more energy, the weight gain of cafeteria-fed rats was only 27% greater than that of controls. The hyperphagia was due to a combination of a greater weight of food eaten per day and the higher energy density of the cafeteria foods, and was still apparent when energy intakes were corrected for differences in body size.

Analysis of body composition revealed that 93% of the excess weight gain of cafeteria rats consisted of fat deposition, resulting in a large increase in body energy content (Table 1). However, the energy cost of weight gain (g gain per MJ eaten) was significantly lower in cafeteria rats (11.23 ± 0.55) than

Table 1 Energy balance data for control and cafeteria rats from day 0 to day 21

	Control	Cafeteria
Body weight gain (g)	103 ± 7	131 ± 8*
Body fat gain (g)	40 ± 3	66 ± 5†
Energy intake (kJ)	6,480 ± 200	11,670 ± 330‡
(kJ/W ^{0.75} /day)	650 ± 10	1,140 ± 30‡
Final body energy (kJ)	4,100 ± 160	4,540 ± 120*
Body energy gain (kJ) (days 0–21)	1,790 ± 160	2,230 ± 130*
Efficiency of gain (kJ gain per kJ eaten)	0.36 ± 0.07	0.22 ± 0.01†
Energy expenditure (kJ) (kJ/W ^{0.75} /day)	4,690 ± 100	9,440 ± 250‡
	470 ± 10	930 ± 20‡

Initial body weight (g) on day 0 was; control 318 ± 4, cafeteria 315 ± 4. Energy intake was estimated from (1) weight of stock diet (PRD, Christopher Hill Group Ltd) consumed and its metabolisable energy density (10.75 kJ per g, previously determined experimentally) and (2) the weight of each cafeteria food item consumed and the metabolisable energy density given in food composition tables²¹. These animals were killed on day 21, body composition determined by analysis and body energy calculated from this using values of 38.0 and 19.16 kJ per g for the energy value of fat and protein respectively²². A group of six animals were also killed on day 0, body energy content determined in the same way and this value subtracted from the final body energy content of the experimental animals in order to estimate body energy gain. The initial body composition of this baseline group and the experimental animals was also determined indirectly on day 0 using an *in vivo* tritium dilution method²³. The composition of both experimental groups was identical and no different from that of baseline animals. Energy expenditure over the 21 days of the experiment was estimated by subtracting the body energy gain from the metabolisable energy intake $W^{0.75}$ = body weight (kg)^{0.75}. Mean values ± s.e.m., n = 6, * P < 0.05, † P < 0.01, ‡ P < 0.001 compared to controls.

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controls (15.89 ± 0.67 , $P < 0.01$) and body energy gain per unit intake was similarly reduced by 39% in experimental animals (Table 1). This contrasts with our report²⁴ that efficiency (g gained per MJ) was not significantly increased in cafeteria rats and may be due to inherent differences in metabolic efficiency between diverse groups of experimental animals. In a recent experiment with the same strain of rat from two different breeding colonies (Tuck, Essex and Charles River, Kent) the efficiency of weight gain of 'Tuck' rats fed the cafeteria diet was 12% greater than the stock fed controls, although not significantly so, whereas the efficiency of 'Charles River' cafeteria animals was 17% lower than their controls ($P < 0.01$). The genetic influence on metabolic efficiency is well known^{25,26}, but the above results illustrate that even within the same strain of rat, small differences in genetic and/or environmental background can exert a profound influence on the metabolic response to overfeeding.

Our studies show some parallel with those carried out in man, particularly the overfeeding experiments¹³⁻¹⁵. In human subjects, changes in the energy cost of weight gain have been ascribed to increases in DIT, and our energy balance results confirm a similar effect in the overfed rat. There was a 100% increase in the total energy expenditure of cafeteria-fed rats, a difference which is still apparent when the results are corrected for the greater body weight of these animals (Table 1). It is unlikely that such large differences in energy expenditure could be accounted for by activity which, at best, represents only a small fraction of energy expenditure in the rat and these adult rats were housed in pairs in cages designed to restrict activity. The differences in daily heat production observed would require prodigious activity (equivalent to each rat walking 6 km per day). It is also unlikely that these differences were due to the energy cost of the excess fat gain in cafeteria rats. Less than 8% of the extra energy expended by cafeteria rats could be accounted for in this way if a value of 14 kJ per g (ref. 27.) is assumed for the energy cost of fat gain.

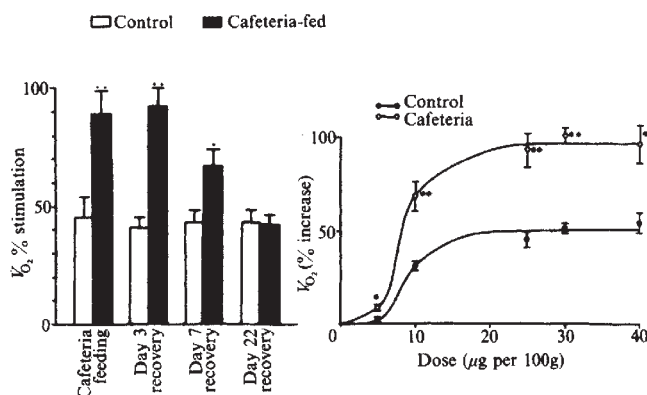


Fig. 1 a, Effect of noradrenaline on oxygen consumption in cafeteria and control animals. Adult, female Sprague-Dawley rats were fed the cafeteria diet for 28 days and noradrenaline sensitivity was measured on day 26. The cafeteria diet was withdrawn on day 28, allowing free access to stock diet and noradrenaline sensitivity was measured 3, 7 and 22 days later. Cafeteria rats were 50 g heavier than controls on day 26 and had completely returned to control weight by day 22 of recovery. Oxygen consumption was measured for 2 h before and after injection of noradrenaline (Levophed, 25 $\mu\text{g per } 100\text{g}$ body weight, s.c.). Results are presented as oxygen consumption after noradrenaline as a percentage of the pre-injection value. Mean values, bar denotes s.e.m. * $P < 0.05$, ** $P < 0.01$, compared to controls. b, Stimulation of resting oxygen consumption by various doses of noradrenaline. Adult female Sprague-Dawley rats were maintained on the cafeteria diet for 15 days and during this time gained 23 g excess weight compared to their free-feeding controls. Two days after withdrawal of the mixed diet resting oxygen consumption was determined for 2 h before and after administration of noradrenaline. One dose of noradrenaline was tested in four control and four experimental animals and the withdrawal of the cafeteria diet was staggered in order to facilitate these measurements. Mean values, bar denotes s.e.m., $n = 4$. * $P < 0.05$, ** $P < 0.01$, compared to controls.

Table 2 Resting oxygen consumption ($\text{ml/min/W}^{0.75}$) of control and experimental rats in response to propranolol, during and after cafeteria feeding

	Control	Cafeteria
<i>Cafeteria feeding period</i>		
Day 5	11.91 ± 0.41	$14.48 \pm 0.16^\dagger$
Day 11	11.74 ± 0.25	$14.11 \pm 0.46^\ddagger$
Day 13	10.82 ± 0.28	$14.05 \pm 0.45^\ddagger$
Day 16	10.65 ± 0.24	$13.65 \pm 0.58^\ddagger$
<i>Propranolol injected (day 13)</i>		
Before	10.82 ± 0.28	14.05 ± 0.45
After	10.85 ± 0.29	11.80 ± 0.26
	NS	++
<i>Recovery period</i>		
Day 1	10.73 ± 0.16	$12.21 \pm 0.38^\ddagger$
Day 2	10.14 ± 0.14	$12.30 \pm 0.23^\ddagger$
Day 3	10.32 ± 0.17	$11.49 \pm 0.41^*$
Day 13	10.61 ± 0.28	$10.50 \pm 0.29(\text{NS})$
<i>Propranolol injected (day 2)</i>		
Before	10.14 ± 0.14	12.30 ± 0.23
After	10.15 ± 0.19	10.88 ± 0.27
	NS	++

Resting oxygen consumption was measured in a closed circuit respirometer²⁸ for 2 h during the day, at a temperature of $29 \pm 1^\circ\text{C}$. On day 13 of cafeteria feeding and day 2 of the recovery period oxygen consumption was measured before and after injection of propranolol (0.5 mg per 100 g body weight s.c.). Resting values were obtained as described in ref. 29 and have been corrected for differences in body size. The same animals were used for these measurements throughout the 21 day cafeteria feeding period and the withdrawal of the mixed foods. Oxygen consumption was determined on the first 3 days of the recovery period since it is during this time that weight loss is most rapid and the measurement on day 13 was made when the body weight of the cafeteria rats had returned to control levels. Mean values $\pm$ s.e.m., $n = 6$: * $P < 0.05$, $^\dagger P < 0.01$, $^\ddagger P < 0.001$, compared to controls: ++ $P < 0.01$ compared to pre-injection value, NS not significant.

The association of lowered efficiency with increased DIT in the overfed animals was confirmed by measuring resting oxygen consumption ($\dot{V}_{O_2}$ (Table 2). Because these represent sample daytime measurements in resting animals they exclude the effects of activity and night-time feeding. Nevertheless, a 20–30% difference in $\dot{V}_{O_2}$ was consistently observed between control and experimental rats throughout the period of weight gain and, confirming our previous findings^{19,20,24}, also during the initial recovery period when cafeteria rats were losing weight rapidly. This sustained increase in DIT after the cafeteria diet was withdrawn is one of the most interesting effects of this treatment since during the recovery period animals are hypophagic^{19,24}. Thermogenesis was then dependent on the degree of obesity and $\dot{V}_{O_2}$ returned to control levels as body weight declined to normal. This independence of DIT from the immediate energy intake indicates a chronic metabolic adaptation to hyperphagia and obesity.

Unlike previous demonstrations of DIT, our results clearly establish the cafeteria system as a method for inducing DIT in normal rats without resorting to nutritionally-unbalanced diets (such as low-protein diets) and we can now consider the metabolic origins of DIT using this model.

Metabolic changes

Increased sensitivity to the thermogenic effects of noradrenaline in cafeteria-fed rats²⁰ is illustrated in Fig. 1. In experimental animals the increase in $\dot{V}_{O_2}$ after noradrenaline was twice that seen in controls, both during the period of weight gain and when cafeteria feeding was stopped, after 3 days of weight loss: these differences diminished as the animals returned to control body weight. In those rats described in Table 1 there was also a greater sensitivity to noradrenaline during the period of weight gain with the control and cafeteria rats showing increases in $\dot{V}_{O_2}$ of 6% and 76% respectively. To confirm that differences in sensitivity to noradrenaline were not a reflection of the dose used, doses of 5–40 $\mu\text{g per } 100\text{g}$ body weight were tested (Fig. 1b). Cafeteria animals were more sensitive throughout the range: response was maximal for approximately the same dose given to both groups (25 $\mu\text{g per } 100\text{g}$), whereas the lowest dose (5 $\mu\text{g per } 100\text{g}$)

100 g) produced a significant response in cafeteria rats only. There was no correlation between noradrenaline sensitivity and body fat content in either group ($r < 0.3$) indicating that the enhanced sensitivity was not simply a consequence of a greater fat mass.

There are many reports of enhanced sensitivity to the thermogenic effects of noradrenaline in animals exhibiting NST and the differences between warm and cold-adapted rats^{30,31} are as great as those seen in control and cafeteria animals. The effects of increased sympathetic activity on oxygen consumption in cold adapted rats can be inhibited using agents that block catecholamine receptors³² and might therefore be expected to have similar effects on DIT. Table 2 shows that the β -adrenergic blocking agent propranolol produced a significant decrease in the $\dot{V}_{O_2}$ of cafeteria rats while on the mixed diet and during the early recovery phase, but had no effect on the oxygen consumption of control animals. The changes in noradrenaline sensitivity and the effects of propranolol on $\dot{V}_{O_2}$ therefore indicate an increase in sympathetic activity in cafeteria-fed animals and suggest that the sympathetic nervous system is involved in the control of DIT as well as NST. Further support for this idea comes from the report by Young and Landsberg³³ of comparable increases in cardiac noradrenaline turnover between overfed rats and rats adapted to the cold.

Such results and the similarities between NST and DIT inevitably lead to the question of whether the same effector tissue is involved in the two types of thermogenesis. As stated above, the main effector tissue for NST is BAT. It has not previously been suggested that BAT plays a thermogenic role in any situation other than cold exposure. However, we have found increased deposits of BAT in the interscapular region of cafeteria rats and this would be consistent with other similarities we have noted between these rats and cold-adapted animals.

Because BAT is highly thermogenic it can be detected, particularly in the interscapular region, using the non-invasive technique of measuring surface temperature³⁴. Surface and rectal

Table 3 Brown adipose tissue weight, composition and *in vitro* release of free fatty acids (FFA)

	Control	Cafeteria
Interscapular BAT weight (mg)	266 ± 13	692 ± 28†
(mg per 100 g body wt)	63 ± 3	156 ± 9†
Interscapular BAT composition		
% Fat	36.3 ± 1.9	36.8 ± 2.3
% Fat-free dry wt	8.7 ± 0.8	9.8 ± 1.1
<i>In vitro</i> FFA release (μM FFA per mg per 2 h)		
Basal	5.54 ± 0.17	5.09 ± 0.29
Noradrenaline stimulated	7.33 ± 0.35	11.70 ± 0.59†
% Increase	33 ± 7	132 ± 15†

The fat content of brown adipose tissue was determined by ether extraction. Basal *in vitro* release of FFA was estimated using duplicate 50 mg samples. Each sample was incubated in 1 ml Earle's bicarbonate (pH 7.4, containing 2% bovine serum albumin), at 37 °C in a shaking water bath for 2 h. Stimulated FFA release was measured in another set of duplicates with noradrenaline (final concentration 2.5 μg ml⁻¹) added to the medium. After incubation the tissue was removed and the FFA content of the medium was determined by a micro-colorimetric method³⁵. Mean values ± s.e.m., $n = 6$, † $P < 0.001$ compared to controls.

temperatures were therefore measured in control and cafeteria rats before and after injection of noradrenaline 25 μg per 100 g (Fig. 2). Skin temperature was measured between the scapulae where large deposits of BAT are seen in cold-adapted rats and on the abdomen directly over the liver. Before injection of noradrenaline mean rectal and interscapular temperatures of cafeteria rats were, respectively, 0.6 and 0.8 °C higher than those of controls ($P < 0.001$) but abdominal temperature was similar in the two groups. Noradrenaline produced a marked rise in skin and rectal temperatures of experimental animals but did not significantly alter those of controls. The interscapular temperature increased by 1.3 °C after noradrenaline compared to only 0.7 °C for the abdomen, so that the interscapular temperature of cafeteria rats was significantly higher than abdominal after noradrenaline ($P < 0.001$). This marked increase in skin temperature over the interscapular BAT in cafeteria rats after noradrenaline indicates either an increase in heat production of this tissue and/or an elevated blood flow, both of which are seen in the cold-adapted rat after noradrenaline⁴.

These animals were killed after 21 days of cafeteria feeding and interscapular BAT was dissected out. The weight of tissue found in cafeteria rats was similar to that found in rats adapted to 5 °C^{30,36} but was more than twice that found in controls (Table 3). The observed increase was due to an increase in active tissue mass rather than excessive lipid deposition (Table 3) suggesting a true hypertrophy in response to cafeteria feeding. Table 3 also shows that although basal release of free fatty acids (FFA) from interscapular BAT was similar for both groups, the stimulated release in the presence of noradrenaline (% increase) was four times greater in cafeteria rats.

Thus by various criteria (interscapular BAT mass, temperature response and lipolytic sensitivity to noradrenaline) it is possible to demonstrate an association between changes in BAT and DIT that resemble the changes seen in cold-adapted animals. The contribution of BAT thermogenesis to total heat production has yet to be quantified but there is a remarkable correlation between resting $\dot{V}_{O_2}$ of cafeteria rats and their interscapular BAT mass ($r = 0.8$, $n = 12$, $P < 0.001$) which is not apparent in control animals ($r = 0.45$, $n = 12$, not significant). If such a relationship were causal it would not necessarily exclude the participation of other tissues in the metabolic response to overfeeding and we have shown that there are increases in the lipolytic sensitivity of white adipose tissue to noradrenaline in cafeteria rats²⁰. Nor can other thermogenic hormones, in particular the thyroid hormones be excluded. The role of the thyroid in NST is unclear (see ref. 1) but injections of thyroid hormones have been shown to induce hypertrophy of BAT, increase noradrenaline sensitivity and improve cold tolerance³⁰. In addition, recent studies have demonstrated that circulating levels of

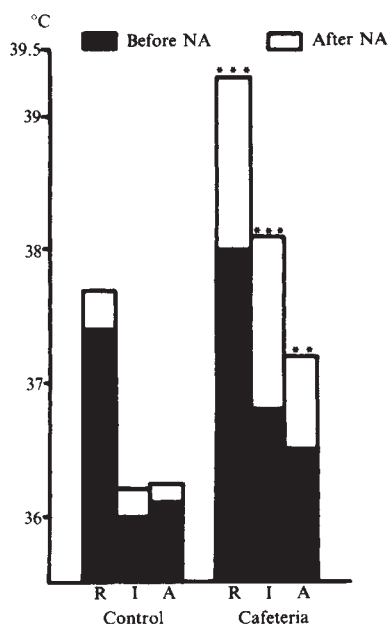


Fig. 2 Rectal (R), interscapular (I) and abdominal (A) temperatures of control and cafeteria rats before and after noradrenaline. Rectal and skin temperatures were measured on day 19 of cafeteria-feeding using thermocouples sensitive to 0.05 °C (Comark 1694 F) in an air-conditioned room at 24 ± 1 °C. Skin temperature was determined between the scapulae and on the abdomen just below the xiphisternum by taping a thermocouple over the surface of the skin and rectal temperature was measured by insertion of a plastic coated thermocouple 4 cm into the animal. Three readings were taken at 5 min intervals for each site before injection of noradrenaline (25 μg per 100 g body weight) and a further three measurements at 5-min intervals were carried out 1.5 h after administration of the drug. Mean values, $n = 6$. ** $P < 0.01$, *** $P < 0.001$, compared to pre-injection temperature.

triiodothyronine (T_3) are elevated in overfed human subjects³⁷ and in animals exhibiting DIT in response to low-protein diets^{11,12}. It is therefore not surprising that plasma T_3 levels were elevated by 27% in cafeteria rats during mixed diet feeding (T_3 (ng ml⁻¹): control 0.89 ± 0.03 , cafeteria 1.14 ± 0.08 , $P < 0.01$). This difference was still apparent 4 days after the mixed diet was withdrawn when cafeteria rats were in fact hypophagic compared to controls but still had a higher oxygen consumption. These higher T_3 levels may not necessarily be directly responsible for the elevated heat production of cafeteria rats but could explain the hypertrophy of BAT and enhanced sensitivity to noradrenaline seen in these animals.

Brown fat in man

We have pointed out³⁸ that obesity induced by cafeteria feeding has several advantages over other experimental models of obesity (such as genetic, hypothalamic and drug-induced obesity). Apart from the fact that cafeteria feeding does not involve pathological and irreversible changes, it is perhaps the closest approximation to the human condition where, at least in some parts of the world, a varied and highly palatable diet encourages over-indulgence. In spite of this, many individuals remain lean, perhaps because they, like the rat, exhibit DIT. One major difference between the rat and man is that although adult man does possess some BAT^{39,40} this was not considered to be thermogenically active. However, the association between DIT and BAT in the rat demands a reconsideration of the role of this tissue in all species, including man, and promoted us to undertake the experiment shown in Table 4. It was postulated that the existence of functional BAT in man could be identified by local changes in skin temperature during stimulation by a sympathomimetic agent such as ephedrine, which increases metabolic rate in man⁴¹. Ephedrine produces the largest increases in skin temperature in the neck and upper back—areas that correspond to BAT locations in the human neonate and adult^{34,39}. The existence of these localised areas which are sensitive to ephedrine has also been confirmed using IR thermography (Fig. 3). These findings can be interpreted as evidence for functional BAT in man.

Conclusions

By adopting the cafeteria feeding system we have been able to demonstrate the existence and importance of DIT in energy metabolism. The striking similarities between DIT and NST suggest that these two phenomena may have a common metabolic origin in BAT. The importance of NST is mainly restricted to cold-adaption in small mammals. The role of BAT in man is not clear, but our findings now raise questions as to the advantages of possessing this energetically wasteful tissue. Confirmation of the thermogenic activity and quantitative

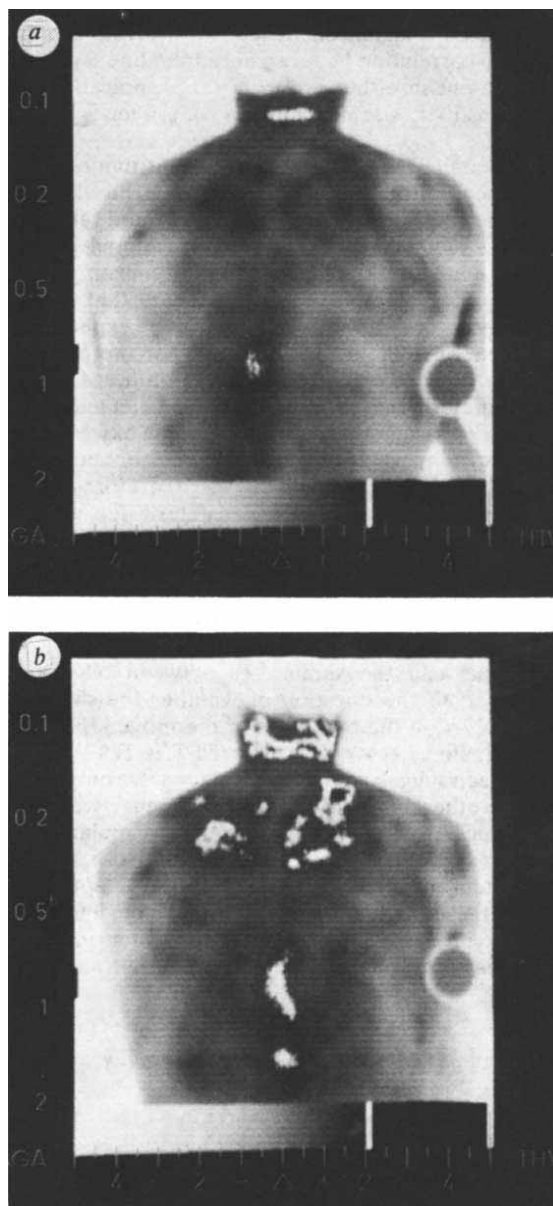


Fig. 3 IR thermograms of one subject before and after administration of ephedrine. IR thermograms (AGA Thermovision; 2–6 μm) were made after the subject had equilibrated for 12 min at a room temperature of 19 °C. Before taking ephedrine (a) the highest skin temperature recorded, which corresponds to the vertical bar on the lower scale, was 34.1 °C. The areas of skin isothermal to this temperature appear as white patches on the image. One hour after taking ephedrine (b) the areas at this temperature have increased but are concentrated between and above the scapulae and in the neck region. The subject was male, aged 36 yr and the dose of ephedrine was the same as in Table 4.

Table 4 Changes in skin temperature (Δ °C) following the oral administration of ephedrine

	Subject A	Subject B	BAT site
Nape of neck	0.4	0.8	+
Interscapular	0.7	0.6	+
Suprascapular	0.4	0.4	NE
Subscapular	0	0.5	NE
Lower spine	0.1	-0.1	NE
Abdomen	-0.1	-0.1	-
Sternum	0.4	0.7	+
Shoulder	-0.3	0.2	NE
Oral	0.1	-0.2	

Skin temperatures were recorded on the unclothed torso before and after taking ephedrine (1 mg per kg body weight), using a multi-channel electronic thermometer (Comark 1604). The skin thermocouples were kept in place for the duration of the experiment which was conducted in a room maintained at 25 °C. Subject A was male, aged 36 yr and subject B was female, aged 23 yr. The symbols in the column headed 'BAT site' refer to BAT locations in adult man as described by Heaton³⁹ (+, positive identification; -, negative; NE, not examined).

significance of BAT in man must await more direct methods for studying local tissue heat production and blood flow but if BAT in adult man has an oxidative capacity similar to that of other species it could make a significant contribution to energy expenditure. Similarly, a defect in, or an absence of BAT would predispose to obesity. The finding⁴² of a decreased thermogenic response to noradrenaline in constitutionally obese humans is similar to that reported in the genetically obese mouse⁶. If, as in the obese mouse, this loss of sensitivity is associated with a defect in BAT⁴³ the importance of this tissue in man will assume clinical significance.

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Expression of the chromosomal mouse β^{maj} -globin gene cloned in SV40

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The complete chromosomal mouse β^{maj} -globin gene, including its intervening and flanking sequences, has been cloned in the monkey virus SV40. The mouse gene is transcribed, processed and translated in infected monkey kidney cells to yield mouse β^{maj} -globin.

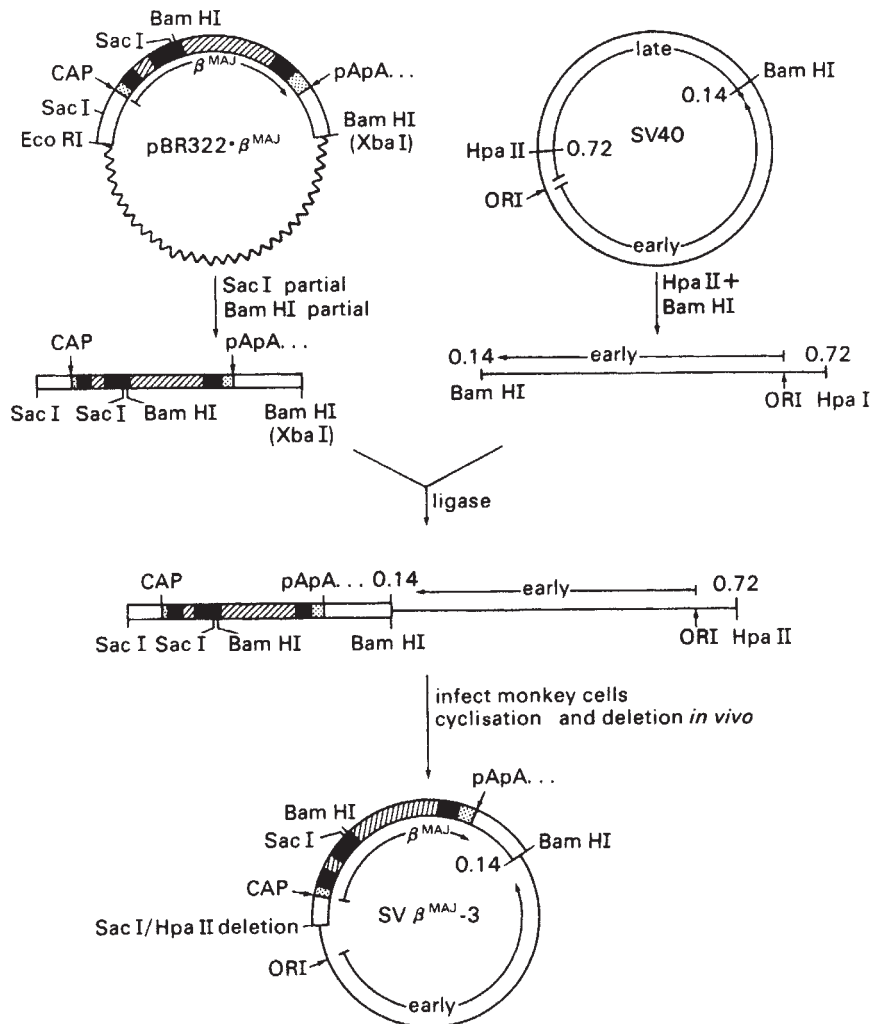
WE have identified several conserved sequences in the chromosomal DNA of cloned mouse α - and β -globin genes that may have an important role in their ordered expression and in the processing of their initial transcripts^{1–5}. Despite the fact that these sequences have been preserved during several hundred million years of separate evolutionary development, we cannot be certain of their function or importance until they, or mutants derived from them, have been tested in an appropriate biological system. Our first approach to the development of such tests has involved the use of animal viruses as vectors of chromosomal genetic information. Here, we have inserted the complete genomic mouse β^{maj} -globin gene into the monkey virus SV40 and used the resulting recombinant to infect cultured monkey kidney cells. Despite the species and cell-type differences, the mouse globin signals for RNA splicing, polyadenylation and translation are recognised in this host, and substantial quantities of mouse β^{maj} -globin are produced. Thus, the SV40-monkey cell system seems to provide a useful and logical extension of the recombinant DNA technology, returning the cloned gene to a cell in which its function can be tested.

The present experiment was based on our previous experience with SV40 hybrids carrying both prokaryotic and eukaryotic DNA fragments. Initially, we used an *Escherichia coli* suppressor tRNA gene to develop the SV40 late region deletion vehicle system. We found that such vehicles could be used to propagate the foreign DNA either as virus in productively infected cells^{6,7}, or as a stable genetic element in transformed or persistently infected cells^{8,9}. Subsequently, we inserted a rabbit β -globin coding sequence, derived from a

cloned reverse transcript, into two different sites in the SV40 late region¹⁰. As anticipated, expression of the cDNA gene into a stable, translatable mRNA required the retention of the appropriate signals for mRNA processing (and possibly translation) in the viral vector. A similar conclusion was reached by Mulligan *et al.*¹¹, who inserted the rabbit sequence at a different site in the viral late region. Most recently, we constructed viruses carrying a portion of the mouse β^{maj} -globin gene, and provided evidence for the use of a globin splice junction and polyadenylation site when the sense strand of the gene was transcribed from the viral late region promoter¹². However, because of the limitations of our biochemical analyses, we could not be certain that the processing reactions were occurring at exactly the appropriate sites. This encouraged us to insert the complete chromosomal gene into SV40, thereby providing a more stringent test for functional mRNA synthesis—translation into authentic globin.

Construction of an SV40 recombinant carrying the complete chromosomal mouse β^{maj} -globin gene

In the recombinant virus described here, the complete chromosomal mouse β^{maj} -globin gene, including its intervening and adjacent flanking sequences, replaces the viral late gene region. The gene is inserted in the same orientation as the SV40 late protein coding sequences. Therefore, readthrough transcription from the strong viral late region promoter, which is preserved in the vector, generates the globin coding sequence rather than its complement. The construction of this recombinant, depicted in Fig. 1, involved four steps: preparation of the appropriate globin and viral vector DNA segments; ligation *in vitro* of these segments to form linear recombinant molecules with the appropriate orientation; cyclisation of the recombinant genomes *in vivo* following DNA infection of cultured monkey kidney cells; and isolation of a clone of virus having the desired structure.



We originally cloned the β^{maj} -globin gene in bacteriophage λ as part of a 7,000-base pair fragment of chromosomal DNA¹. Complete nucleotide sequence analysis of this gene revealed that it is divided into three discontinuous coding segments by two intervening sequences of DNA⁵. The shorter intervening sequence is 116 base pairs long and interrupts the gene between amino acid codons 30 and 31, whereas the longer is 646 base pairs and interrupts between amino acid codons 104 and 105. Because the maximum amount of DNA which can be inserted in the SV40 late region is only 2,500 base pairs, it was not possible to use the whole 7,000-base pair globin fragment. Instead, we prepared a 2,400-base pair subfragment extending from a *Sac*I site, located 430 base pairs upstream from the 5'-end of the globin mRNA sequence, to an *Xba*I site, located 600 base pairs downstream from the 3'-end of the mRNA sequence. This fragment contains, in addition to the entire globin coding sequence, the sequences adjacent to the sites at which the cap is added to the 5'-end and poly(A) to the 3'-end of the globin mRNA. It also contains two RNA splice junctions and their corresponding intervening sequences. To facilitate the preparation of this fragment and its cloning in SV40, we converted the *Xba*I site to a *Bam*HI site by ligation to a synthetic oligonucleotide linker, then subcloned a restriction fragment containing the relevant sequences in a plasmid vector.

The viral vector segment, which was prepared by cleavage of wild-type SV40 DNA with *Bam*HI at map position 0.14 and *Hpa*II at map position 0.72, retains the viral origin of replication, the entire early gene region and the sequences corresponding to the extreme 5'- and 3'-termini of the late 16S and 19S mRNAs. It lacks the coding sequences for the viral late proteins VP1, VP2 and VP3 and the splice junctions for their corresponding mRNAs¹³⁻¹⁶. We have previously demonstrated that various foreign DNA fragments inserted in this vector are efficiently transcribed from the SV40 late promoter, but the resulting transcripts are not appropriately processed and are rapidly degraded in the cell¹⁰. This might be because the viral late mRNA splice junctions are absent from the hybrid virus. Recombinants formed with this vector lack the viral late genes and are therefore defective. However, because the recombinants retain a functional early gene region, they can be propagated by co-infection with a temperature-sensitive early gene mutant virus as helper^{6,7,34}. When such mixed infections are carried out at the non-permissive temperature, progeny virus is produced only by those cells infected with both the recombinant, which provides functional early gene product, and the helper, which provides late gene products.

The globin and viral DNA segments, both of which had one cohesive *Bam*HI terminus, were joined by treatment with DNA

Fig. 1 Construction and propagation of the SV40-mouse β^{maj} -globin gene recombinant. Open bars: mouse β^{maj} -globin gene flanking sequences; stippled bars: globin transcribed, untranslated sequences; solid bars: globin coding sequences; hatched bars: globin intervening sequences; wavy lines: plasmid sequences; solid lines: SV40 sequences. The methods used for DNA preparation, restriction endonuclease cleavage, isolation of fragments on gels, ligation, DNA infection of African green-monkey kidney cells and preparation of virus stocks have been described elsewhere^{6,10,12}. **The chromosomal β^{maj} -globin gene fragment:** The upper left diagram shows the structure of pBR322- β^{maj} , which contains the mouse chromosomal β^{maj} -globin DNA sequences from an *Eco*RI site located 1,200 base pairs upstream from the globin cap site to an *Xba*I site located 600 base pairs downstream from the poly(A) addition site. This plasmid was derived from the original recombinant pMB9- β^{maj} (ref. 2) by linking the *Xba*I site to a synthetic dodecamer containing a *Bam*HI site, cloning the resulting *Bam*HI-*Bam*HI fragment in pBR322 (ref. 26), then reconstituting the gene by insertion of the 5'-*Eco*RI-*Bam*HI globin gene fragment. This plasmid was partially digested with *Sac*I and *Bam*HI to generate the 2,400-base pair fragment that was cloned in SV40. **The SV40 vector:** The upper right diagram shows the structure of wild-type SV40 DNA including the direction of transcription of the late and early mRNAs, the origin of viral DNA replication (ORI), and the cleavage sites for *Bam*HI and *Hpa*II relative to the *Eco*RI site at 0.00/1.00 map units^{27,28}. The DNA was cleaved with *Bam*HI and *Hpa*II to generate the 3,000-base pair vector fragment. This fragment retains the entire early region, the origin of replication and the sequences corresponding to the 5'- and 3'-termini of the viral late mRNAs; it lacks the coding sequences for the late viral proteins VP1, VP2 and VP3. **The recombinant molecule:** A mixture containing 24 μ g of the globin gene fragment and 18 μ g of the SV40 vector fragment was treated with DNA ligase, then electrophoresed through a 1% agarose gel to purify the 5,400-base pair linear recombinant genome. This molecule, formed by joining the two *Bam*HI termini, contains the globin coding sequences on the same strand normally occupied by the viral late gene coding sequences. **Propagation:** Approximately 10 ng of the purified recombinant DNA mixed with 70 ng of DNA from the temperature-sensitive early gene mutant helper tsA₂₃₉ and used in a DNA plaque infection of monkey kidney cells at 41°C. Fifty complementation plaques were picked and used to prepare virus stocks. DNAs from these stocks were immobilised on nitrocellulose filters and tested for hybridisation to a probe containing globin gene 5'-flanking sequences (see Fig. 2 legend). Of three isolates showing unequivocal hybridisation, one was kept for further analysis and designated SV β^{maj} -3.

ligase to form a linear recombinant molecule that was purified by electrophoresis in an agarose gel. As shown in Fig. 1, the globin gene could join the vector in only one orientation, such that the globin coding sequence is on the strand normally occupied by the viral late protein coding sequences. The resultant recombinant molecule is about 150 base pairs longer than wild-type DNA and has non-complementary termini. However, previous experiments indicated that such molecules could cyclise *in vivo*, and that this is frequently accompanied by the generation of deletions around the junction between the viral and foreign DNAs (D.H.H., unpublished results). Accordingly, the recombinant DNA preparation was mixed with DNA from a temperature-sensitive early gene mutant as helper, and used in a DNA infection of cultured kidney cells. As anticipated, this mixed infection yielded a substantial number of complementation plaques, whereas infection with equivalent amounts of the recombinant or helper DNAs alone did not.

To identify a clone of virus having the desired structure, we prepared virus stocks from these plaques and tested their DNA for hybridisation to a globin probe containing the globin mRNA cap site and the 5'-genomic flanking sequences (see Fig. 2). Of the first 50 plaques tested, three showed unequivocal hybridisation. Presumably, the others had either suffered deletions extending past the 5'-end of the globin gene, or contained only low levels of recombinant virus. One of the positive clones, designated SV $\beta^{\text{maj-3}}$, was kept for further analysis. This hybrid multiplies quite efficiently in monkey kidney cells. In our standard conditions of infection, the ratio of intracellular recombinant to helper genome is about 1:1.

An analysis of SV $\beta^{\text{maj-3}}$ by restriction endonuclease cleavage and gel blot hybridisation (Fig. 2) confirmed that it had the

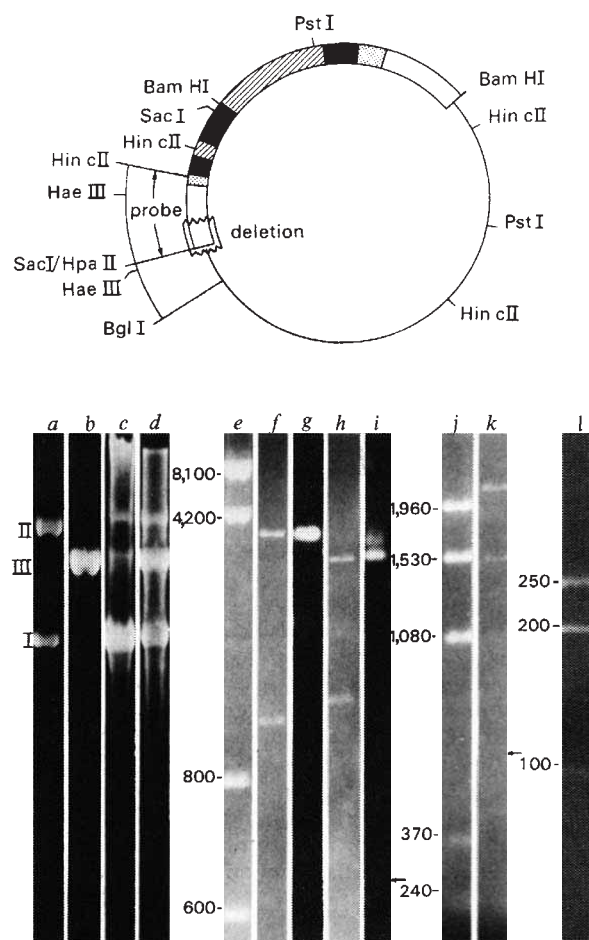
structure expected from its manner of construction and selection. Although the recombinant has the same length as the helper, we were able to purify its DNA because it contains no *EcoRI* sites and one *SacI* site, whereas the helper contains one *EcoRI* site and no *SacI* sites. By digestion with the appropriate endonucleases, we showed that the recombinant has deleted 150 base pairs from the 5'-end of the original globin fragment, leaving 280 base pairs of genomic flanking sequence 5' to the mRNA cap site. Less than 14 base pairs are deleted from the SV40 vector, leaving the predominant viral late mRNA cap sites intact. Thus, SV $\beta^{\text{maj-3}}$ contains the complete mouse β^{maj} -globin gene in the sense orientation relative to the viral late region promoter.

Transcription and processing of globin mRNA

Monkey cells infected with SV $\beta^{\text{maj-3}}$ efficiently synthesise a stable globin mRNA that is spliced and polyadenylated at the appropriate globin gene sites. However, most of this RNA is initiated at the SV40 late promoter region rather than the globin promoter. Evidence for this is derived from gel transfer hybridisation experiments and the translation studies described below.

To characterise the globin RNA made in infected monkey cells, poly(A)-containing RNA was purified^{13,14}, denatured¹⁵, electrophoresed through an agarose gel, transferred and covalently attached to diazobenzoyloxymethyl paper¹⁶, then hybridised with various ³²P-labelled DNA probes to reveal the positions of the separated RNAs (Fig. 3). Hybridisation with total SV40 DNA as probe yielded two bands corresponding to

Fig. 2 Structural analysis of the SV40-mouse β^{maj} -globin gene recombinant. The upper diagram is a restriction endonuclease cleavage map of the recombinant virus SV $\beta^{\text{maj-3}}$. Solid lines: SV40 sequences; open bars: globin gene flanking sequences; stippled bars: globin gene transcribed, untranslated sequences; solid bars: globin gene coding sequences; hatched bars: globin gene intervening sequences. The bottom panels show an analysis of the recombinant virus by restriction endonuclease cleavage and gel transfer hybridisation. A mixture of SV $\beta^{\text{maj-3}}$ recombinant plus *tsA₂₃₉* helper DNA was prepared by the method of Hirt²⁹ from monkey kidney cells that had been infected with the recombinant plus helper stock for 48 h at 40 °C. To prepare pure SV $\beta^{\text{maj-3}}$ DNA, this mixture was digested with *EcoRI*, which has one site in the helper but none in the recombinant, and the resistant recombinant genomes were recovered as covalently closed circles as shown in track *d*. This preparation was digested with *SacI*, which has one site in the recombinant but none in the helper, and the linear recombinant molecules were further purified by electrophoresis through a 1% agarose gel. A fragment containing the deleted junction between the globin and SV40 fragments was prepared by digestion with *HincII* plus *BglI* as shown in the diagram and electrophoresis of the resulting fragments through a 2% agarose gel. Tracks *a-d* were electrophoresed through 1% agarose gels, *e-i* through 1.4% agarose gels, *j, k* through 2% agarose gels, and *l* through an 8% acrylamide gel. Tracks *a-f*, *h* and *j-l* are photographs of ethidium bromide-stained gels. Tracks *g* and *i* are autoradiographs of nitrocellulose transfer strips that were hybridised to a pMB9- β G2/*SacI*-*HincII* probe containing flanking and transcribed, untranslated sequences from the 5'-portion of the globin gene (see upper diagram). Each track represents the following: *a*, undigested wild-type SV40 DNA. The positions of covalently closed circular (form I), nicked circular (form II) and linear (form III) SV40 are shown to the left; *b*, *EcoRI* digest of wild-type SV40 DNA gives linear molecules; *c*, undigested SV $\beta^{\text{maj-3}}$ plus *tsA₂₃₉* DNA; *d*, *EcoRI* digest of SV $\beta^{\text{maj-3}}$ plus *tsA₂₃₉* DNA gives about 50% resistant form I recombinant molecules and 50% sensitive form III helper molecules; *e*, *SacI* plus *PstI* digest of pMB9- β G2 DNA gives marker fragments with lengths (in base pairs) shown to the left; *f*, *BamHI* digest of SV $\beta^{\text{maj-3}}$ /*SacI* DNA gives 3,700- and 1,500-base pair fragments; *g*, transfer of track *f* showing hybridisation to 3,700-base pair fragment; *h*, *PstI* digest of SV $\beta^{\text{maj-3}}$ /*SacI* DNA gives 3,000-, 1,570- and 630-base pair fragments. The position of the 630-base pair fragment, which can be seen only faintly in this reproduction, is indicated by the arrow to the right; *i*, transfer of track *h* showing hybridisation to 3,000-base pair fragment; *j*, *HincII* digest of wild-type SV40 DNA gives marker fragments with lengths (in base pairs) shown to the left; *k*, *HincII* digest of SV $\beta^{\text{maj-3}}$ /*SacI* DNA gives fragments of 2,100, 1,530, 1,080 and 580 base pairs. The position of the 580-base pair fragment, which can be seen only faintly in this reproduction, is indicated by the arrow to the right; *l*, *HaeIII* digest of the SV $\beta^{\text{maj-3}}$ /*HincII*-*BglI* junction fragment gives three fragments with the lengths (in base pairs) shown to the left. The lengths of these fragments were estimated by their mobility relative to several sequenced SV40 and globin gene fragments (not shown). There are single *HaeIII* sites in the globin gene sequence between the *HincII* site and the deleted *SacI* site and in the SV40 sequence between the *BglI* site and the deleted *HpaII* site. Because *HaeIII* digestion of the junction fragment yields three fragments, both *HaeIII* sites must be preserved. The *HaeIII* site in SV40 is 15 base pairs from the *HpaII* site. Therefore, the deletion must cover less than 14 base pairs of SV40 sequence and 150-164 base pairs of globin gene flanking sequence.



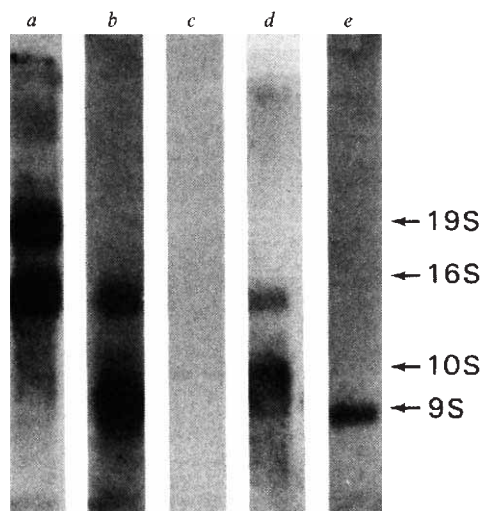


Fig. 3 Gel transfer hybridisation analysis of the globin RNA made in monkey cells infected with the SV40-mouse β^{maj} -globin gene recombinant. **RNAs and hybridisation:** A confluent monolayer of about 2×10^7 monkey kidney cells was infected with 5 ml of the SV β^{maj} -3 plus αS_{239} stock and incubated for 48 h at 40 °C. Total cell poly(A)-containing RNA was prepared by extraction with hot phenol¹³ and oligo(dT)-cellulose chromatography¹⁴, denatured with glyoxal¹⁵, electrophoresed through a 1.5% agarose gel and transferred to diazobenzoyloxymethyl paper¹⁶. Each lane contains 10% of the total sample. The resulting imprints were preincubated, hybridised with approximately 0.1 ng of ^{32}P -labelled, nick-translated DNA probe (25–50 c.p.m. per pg), washed and autoradiographed^{10,16}. Globin mRNA was prepared from reticulocytes obtained from anaemic mice. **Probes** (see maps, Figs 1, 2): The globin probes were prepared by cleavage of the plasmid pMB9-MBG2, which contains the entire 7,000-base pair chromosomal mouse β^{maj} -globin fragment². The globin coding sequence probe contained 900 base pairs extending from the *Pst*I site 75 base pairs upstream from the 3'-coding segment to the *Xba*I site 600 base pairs downstream from the poly(A) addition site. The globin intervening sequence probe contained 600 base pairs extending from the *Bam*HI site 18 base pairs upstream from the large intervening sequence to the *Pst*I site 75 base pairs upstream from the 3'-coding segment. Control experiments with globin mRNA show that hybridisation of the 18 base pairs of coding sequence included in this intervening sequence fragment would not be detected in our conditions of hybridisation, washing and autoradiography. Wild-type SV40 DNA was prepared as described previously⁶. The SV40 late mRNA leader probe contained 250 base pairs extending clockwise from the *Bgl*II site at 0.67 map units in the *Hpa*II site at 0.72 map units. Each track represents the following: a, SV β^{maj} -3 RNA hybridised with total SV40 DNA probe; b, SV β^{maj} -3 RNA hybridised with globin coding sequence probe; c, SV β^{maj} -3 RNA hybridised with globin intervening sequence probe; d, SV β^{maj} -3 RNA hybridised with SV40 late mRNA leader sequence probe; e, globin mRNA (10 ng) hybridised with globin coding sequence probe.

the viral late 16S and 19S mRNAs, presumably due to transcription of the helper virus. When a fragment containing globin coding sequences was used as the probe, we detected a strong globin RNA band at approximately 10S, indicating molecules up to 300 bases longer than authentic globin mRNA (800 bases). This RNA did not hybridise with a probe derived from the large globin intervening sequence, indicating that it has been eliminated and that the second globin splice junction functioned properly in the infected cells. Because the globin RNA is efficiently translated into mouse β^{maj} -globin (see below), it is clear that the first splice junction is also functional and that both splicing reactions are accurate. To determine whether this RNA was initiated at the globin gene promoter or at the upstream viral promoter, we used as probe an SV40 fragment containing the viral late mRNA leader sequences. This probe hybridised strongly to the ~10S globin RNA band, indicating that most of these molecules arise from transcription initiation at the viral promoter. This may also explain the length heterogeneity of the globin RNA as the 5'-viral leader segments are known to be quite variable in length^{17,18}. The SV40 leader probe also detected a 16S band and a very faintly hybridising 19S band (not visible in the reproduction of Fig. 3, lane d) which probably represents helper virus transcription.

In addition to the ~10S globin RNA, using the coding sequence probe we routinely detected a 16S (1,600 base) band

comprising about 15% of the total globin-specific RNA. This RNA is at least partially spliced as shown by its failure to hybridise to the globin large intervening sequence probe. We suspect, but have not proved, that this species results from readthrough past the globin polyadenylation site to the downstream viral polyadenylation site. A similar situation was encountered in our more detailed studies of a recombinant carrying a segment from the 3'-portion of the mouse β^{maj} gene¹².

Judging from the intensities of the bands shown in Fig. 3, we estimate that the steady-state level of the mouse globin RNA produced in monkey cells compares favourably with that of viral late mRNA. Assuming that the globin and late viral mRNAs are initiated at the same rates from the SV40 late promoter, this indicates that the globin RNA is as stable as late viral mRNA. We estimate that approximately 5% of the total cell poly(A)-containing RNA is globin RNA, representing roughly 200,000 molecules per cell.

Mouse β^{maj} -globin is synthesised by infected monkey cells

Monkey kidney cells infected with SV β^{maj} -3 synthesise a protein which we have identified as mouse β^{maj} -globin by immunoprecipitation, two-dimensional electrophoresis and tryptic fingerprint analysis. These tests indicate that the mouse globin mRNA is accurately spliced in monkey cells, and that the mouse gene translational signals are functional.

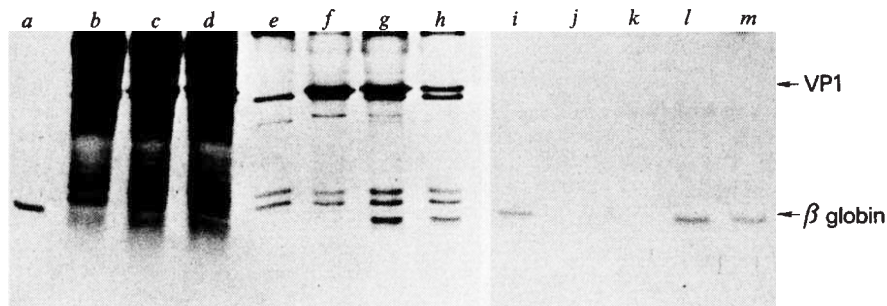
Figure 4 (lanes a–h) shows an experiment in which monkey cells were infected with either the SV β^{maj} -3 plus helper stock or with no virus or wild-type SV40 as controls, incubated until late in the lytic cycle, then labelled for 1 h with ^{35}S -methionine. Sonic extracts were prepared, and a portion of each was electrophoresed through a polyacrylamide-SDS gel, which separates proteins according to their molecular weight¹⁹. As might have been anticipated from the fact that SV40 does not shut down host cell protein synthesis, the resulting autoradiograms showed a complex variety of proteins in which the globin region of the gel was obscured by host bands. However, when the cell proteins were first immunoprecipitated with purified goat anti-mouse β -globin antibody, we detected a band with the same mobility as authentic mouse globin in the SV β^{maj} -3-infected cell extract but not in the mock or wild-type SV40-infected cell extracts. (The additional bands seen in these lanes were also seen using anti-SV40 T-antigen serum, and apparently result from artefactual trapping during the immunoprecipitation procedure.) The exact co-migration of this band with authentic mouse β^{maj} -globin was confirmed by a mixing experiment (Fig. 4, lane h).

The identity of this protein as mouse β^{maj} -globin is supported by a two-dimensional electrophoresis experiment (Fig. 4, lanes i–m). Extracts were prepared and immunoprecipitated as described above, unlabelled mouse globin was added to the eluted proteins and the mixtures were submitted to non-equilibrium pH gradient gel electrophoresis, which separates proteins according to their isoelectric points²⁰. When the proteins co-migrating with mouse β^{maj} -globin, identified by staining, were eluted and electrophoresed through a polyacrylamide-SDS gel, we detected a strong globin band in the SV β^{maj} -3-infected preparation but not in the mock or wild-type SV40-infected preparations. A mixing experiment (Fig. 4, lane m) confirmed the co-migration of this band with authentic globin. Note that the non-equilibrium pH gradient gels used in this experiment are capable of separating proteins that differ by only a single charge unit²⁰.

Tryptic fingerprint analysis provided further evidence that the protein encoded by SV β^{maj} -3 is mouse β^{maj} -globin. The globin band from SV β^{maj} -3-infected cells was purified by immunoprecipitation and polyacrylamide-SDS gel electrophoresis, digested with trypsin and submitted to two-dimensional chromatography on a cellulose thin layer plate^{21,22}. The resulting autoradiogram (Fig. 5) showed two spots with the same relative positions as those found in a digest of authentic mouse

Fig. 4 Production of mouse β^{maj} -globin in monkey cells infected with the SV40-mouse β^{maj} -globin gene recombinant. **Proteins:** Confluent monolayers of about 2×10^7 monkey cells were treated with 1 ml of medium, wild-type SV40 or the SV β^{maj} -3 plus $tS_{A_{239}}$ stock and incubated for 48 h at 40 °C. The cells were washed three times with methionine-free medium, then labelled for 1 h with 200 μCi of ^{35}S -methionine (516 Ci mmol $^{-1}$, NEN) in 1 ml of methionine-free media. After washing twice in ice-cold phosphate-buffered saline (PBS), the cells were scraped off the plates in PBS, collected by centrifugation, resuspended in 0.8 ml of PBS containing 1% Triton X-100 and 0.5% sodium deoxycholate, sonicated for 1 min at full power in a Heat Systems sonicator, and centrifuged at 12,000g for 30 min.

The supernatants were recovered and analysed as described below. To prepare authentic mouse β^{maj} -globin, Friend erythroleukaemia cells were induced with 1% dimethyl sulphoxide for 3 d, labelled with 25 $\mu\text{Ci ml}^{-1}$ ^{35}S -methionine for 12 h, then lysed by freeze-thawing. Haemoglobin was purified on a carboxymethyl cellulose column and converted to globin by precipitation with 2% HCl in acetone³⁰. Pure β^{maj} -globin chains were prepared by carboxymethyl cellulose column chromatography in the presence of 8 M urea³¹. **Immunoprecipitation:** 10 μl of heparin (10 mg ml $^{-1}$), 10 μl of normal goat serum and 50 μl of a 10% suspension of heat-killed formalin-fixed *Staphylococcus aureus* Cowen I bacteria were added to 500 μl of cell extract³². Following 30 min on ice, the bacteria were removed by centrifugation, the supernatant was added to a predetermined excess of purified goat anti-mouse β -globin antibody and incubated 12 h at 4 °C. Then, 50 μl of *S. aureus* suspension was added, the mixtures were kept at 4 °C for 1 h and the bacteria collected by centrifugation. The pellet was washed three times in PBS containing 1% Triton X-100 and 0.5% deoxycholate. A 20% aliquot of each sample was resuspended in 25 μl of SDS sample buffer¹⁹, boiled for 5 min, centrifuged to remove the bacteria, then analysed by polyacrylamide-SDS gel electrophoresis. The remainder of each sample was resuspended in 100 μl of non-equilibrium pH gradient electrophoresis gel sample buffer²⁰, mixed with 5 μg of unlabelled mouse globins, incubated for 1 h at room temperature, centrifuged to remove the bacteria, then analysed by two-dimensional electrophoresis. **Gel electrophoresis:** 20% polyacrylamide-SDS gels were prepared, run and fluorographed as described elsewhere^{19,33}. Non-equilibrium pH gradient gels²⁰ were prepared in 0.6 $\times$ 10 cm tubes and electrophoresed at 400 V (constant voltage) for 3 h. The gels were soaked in 50% methanol for 24 h, stained in 0.01% Coomassie brilliant blue, 50% methanol and 12.5% acetic acid for 15 min, then destained in 7% methanol and 1.25% acetic acid for 18 h. The region of the gel containing mouse β^{maj} -globin (well separated from β^{min} - and α -globin) was excised and the proteins eluted by electrophoresis, precipitated with 2% HCl in acetone, resuspended in 50 μl of SDS sample buffer, boiled for 5 min and electrophoresed through a polyacrylamide-SDS gel. **Quantification of globin synthesis:** 0.67% of the trichloroacetic acid-precipitable radioactivity in the extract from SV β^{maj} -3-infected cells was recovered in the immunoprecipitate. Of this, 15% was in the globin band as determined by densitometry of the autoradiogram (track g). Thus, $0.67\% \times 15\% = 0.1\%$ of the ^{35}S -methionine incorporated during the 1-h pulse was found in globin. Tracks a-h are one-dimensional, polyacrylamide-SDS gel analyses of total cell extracts and immunoprecipitates. Tracks i-m are the second dimension from the two-dimensional analyses (non-equilibrium pH gradient gel electrophoresis followed by polyacrylamide-SDS gel electrophoresis) and were run on a separate polyacrylamide-SDS gel. Each track represents the following a, mouse β^{maj} -globin; b, total mock-infected cell extract; c, total wild-type SV40-infected cell extract; d, total SV β^{maj} -3-infected cell extract; e, immunoprecipitate of mock-infected cell extract; f, immunoprecipitate of wild-type SV40-infected cell extract; g, immunoprecipitate of SV β^{maj} -3-infected cell extract; h, mixture of mouse β^{maj} -globin and immunoprecipitate of SV β^{maj} -3-infected cell extract (one-half the amounts applied to tracks a and g were mixed after immunoprecipitation and elution); i, mouse β^{maj} -globin; j, mock-infected; k, wild-type SV40-infected; l, SV β^{maj} -3-infected; m, mixture of mouse β^{maj} -globin and SV β^{maj} -3-infected samples (one-half the amounts applied to tracks i and l were mixed after immunoprecipitation elution).



β^{maj} -globin prepared from induced Friend erythroleukaemia cells. The identity of the peptides from the SV β^{maj} -3 protein with authentic β^{maj} -globin was confirmed by fingerprinting a mixture containing equal amounts of radioactivity from both samples. Presumably, the two spots detected represent the two internal methionine-containing tryptic peptides of β^{maj} -globin²³. The globin gene also encodes an N-terminal methionine which is removed in globin-producing cells. Because only the expected two spots were observed in the SV β^{maj} -3 protein digest, we conclude that the monkey cells have appropriately removed the N-terminal methionine. Note also that one of the methionine-containing peptides (Leu 105–Lys 121) lies within the third coding sequence immediately after the second splice junction (Arg 104–Leu 105). This provides further evidence for accurate splicing of the mouse globin mRNA in monkey cells.

We estimate that the mouse β^{maj} -globin accounts for approximately 0.1% of the ^{35}S -methionine incorporated during a 1-h pulse (see Fig. 4 legend). This is close to the synthesis rates for the viral proteins VP2 and VP3. Thus, the globin mRNA seems to be translated about as efficiently as viral mRNA in infected monkey kidney cells.

Distribution of RNA processing enzymes

Although the ubiquity of the enzymes and factors involved in translation has long been recognised, relatively little is known about the species and cell-type distribution of RNA processing enzymes. Our previous data¹² and experiments presented here establish that monkey kidney cells can both splice and polyadenylate mouse globin RNA. Although we have not characterised the polyadenylation reaction in detail, we believe that the splicing reaction is both specific and efficient. This is indicated by the translation of the resulting mRNA into β^{maj} -globin, and by the failure to observe accumulation of unspliced precursor molecules.

Because mice and monkeys diverged over 70 Myr ago, and because kidney fibroblast cells are not in the erythroid cell

lineage, we suspect that the RNA splicing and polyadenylation signals are widely recognised among mammalian species and cell types. Our findings are inconsistent with the notion that the differentiation of a globin-producing cell requires the production of enzymes that specifically process globin and other erythroid mRNAs. On the other hand, the results support the validity of comparing the sequences of globin genes from divergent organisms so as to define the nucleotide sequence specificity of RNA processing enzymes.

SV40 transducing viruses as genetic tools

We have established that a mammalian chromosomal gene can be expressed in a foreign cell after cloning in SV40. The chief difference between this and earlier experiments by Mulligan *et al.*¹¹ and ourselves¹⁰ using cDNA recombinants is that here expression of the globin gene depends on the mouse genomic signals for RNA splicing and polyadenylation rather than on the corresponding viral signals. Hence, it seems to be a useful substrate for determining the effects of nucleotide sequence alterations on RNA processing. Further, although we have used the SV40 late promoter in this recombinant, it should be possible to bring the globin gene under the control of its own promoter by inverting the mouse fragment relative to the viral vector.

Although cell transformation techniques may also be useful for globin gene transfer experiments (refs 24, 25 and R. Axel, personal communication), there are several advantages of SV40 as a vector for this purpose. First, its small size simplifies localised biochemical mutagenesis. Second, it replicates efficiently, reaching levels up to 100,000 copies per cell late in infection, thus simplifying the detection of gene products encoded by the transduced DNA. Finally, by placing the genomic fragment under the optional control of the strong viral late region promoter, transcription of the inserted DNA can be assured even if its own promoter is not functional. The system's major limitation is that at most 2,500 base pairs of foreign DNA

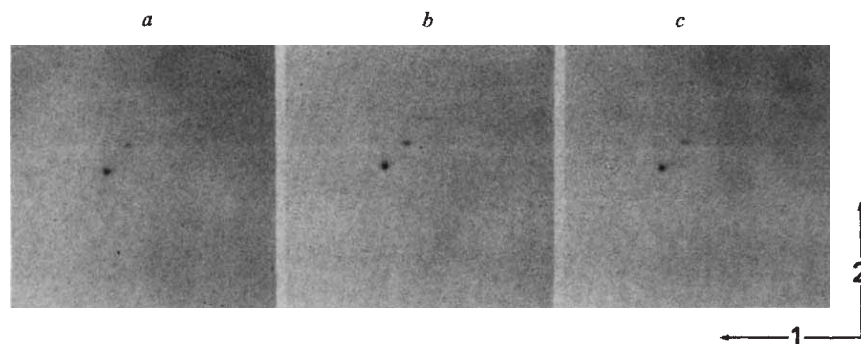


Fig. 5 Fingerprints of tryptic peptides. A 1-ml aliquot of SV β^{ma} -3-infected cell extract was immunoprecipitated as described in Fig. 4 legend, eluted in SDS sample buffer, mixed with 5 μg of unlabelled mouse globin and electrophoresed through a 20% polyacrylamide-SDS gel. A sample of authentic ^{35}S -methionine-labelled mouse β^{ma} -globin from induced Friend cells was electrophoresed in a separate track. The globin bands were identified by staining, excised, washed with 10% methanol, then digested *in situ* with trypsin-TPCK (Worthington)²¹. The supernatants were lyophilised, resuspended in 25 μl of H_2O and analysed by two-dimensional ascending chromatography on $20 \times 20 \text{ cm}$ cellulose thin-layer plates (Brinkman). The first solvent was isoamyl alcohol/pyridine/ H_2O (90:120:105)²². The second solvent was butanol/pyridine/acetic acid/ H_2O (32.5:25:5:20) containing 7% (w/v) 2,5-diphenyloxazole²¹. The autoradiographs were exposed for 8 d at -70°C . *a*, Globin from SV β^{ma} -3-infected cells; *b*, authentic mouse β^{ma} -globin; *c*, mixture of globin from SV β^{ma} -infected cells and authentic mouse β^{ma} -globin (one-half the amounts used in *a* and *b*).

can be inserted into the late region of the recombinant if it is to be encapsidated into virions. This obstacle might be overcome by propagating the hybrid genome as an episome in persistently infected monkey cells⁹, thus overcoming the requirement for encapsidation. Furthermore, it is clear that, although we have

concentrated on the globin system, the approach should also be applicable to a variety of other genes.

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Rabbit β -globin mRNA production in mouse L cells transformed with cloned rabbit β -globin chromosomal DNA

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Mouse thymidine kinase-negative L cells were transformed with a cloned rabbit chromosomal β -globin gene linked to the cloned thymidine kinase gene of herpes simplex virus type 1. Most thymidine kinase-positive cell lines contained one or more copies of rabbit β -globin DNA and produced up to 2,000 copies of rabbit β -globin RNA per cell indistinguishable from its authentic counterpart. No mouse β -globin mRNA was detected.

RECOMBINANT DNA technology has made it possible to isolate, amplify and determine the primary structure of any eukaryotic DNA segment of interest. To explore the functional

significance of particular regions of a genome, it is desirable to generate localised modifications in the nucleic acid and then evaluate the resulting changes in the biological properties of the genome, *in vitro* or *in vivo* (see ref. 1). The reintroduction of defined nucleic acid segments, in particular of cloned DNA, into eukaryotic cells has been accomplished by various methods. Mechanical microinjection of RNA or DNA into oocytes^{2,3} or into somatic cells⁴ delivers a large dose of nucleic acid to a relatively small number of identifiable cells. Biophysical and biochemical approaches use liposomes^{5,6} or erythrocytes⁷ loaded with RNA which are fused with the target cells, or direct treatment of target cells with nucleic acid in the presence of facilitating agents^{8–11}. Efficient delivery of DNA to a large population of cells is attained by the use of DNA incorporated into viral particles¹².

When a high proportion of the treated cells take up and retain the administered DNA the experiment can be evaluated by assaying the entire cell sample; this is the case when oocytes are individually injected, or when cells are infected with viral particles containing a hybrid SV40 genome. Where the foreign DNA transforms only a minute proportion of the cells, as is the case in

most procedures involving administration of exogenous naked DNA, a selection procedure must be used to enrich for transformed cells.

Thymidine kinase-negative cells acquire thymidine kinase activity after transformation with herpes virus DNA, or even better, with an appropriate, purified restriction fragment⁹. As

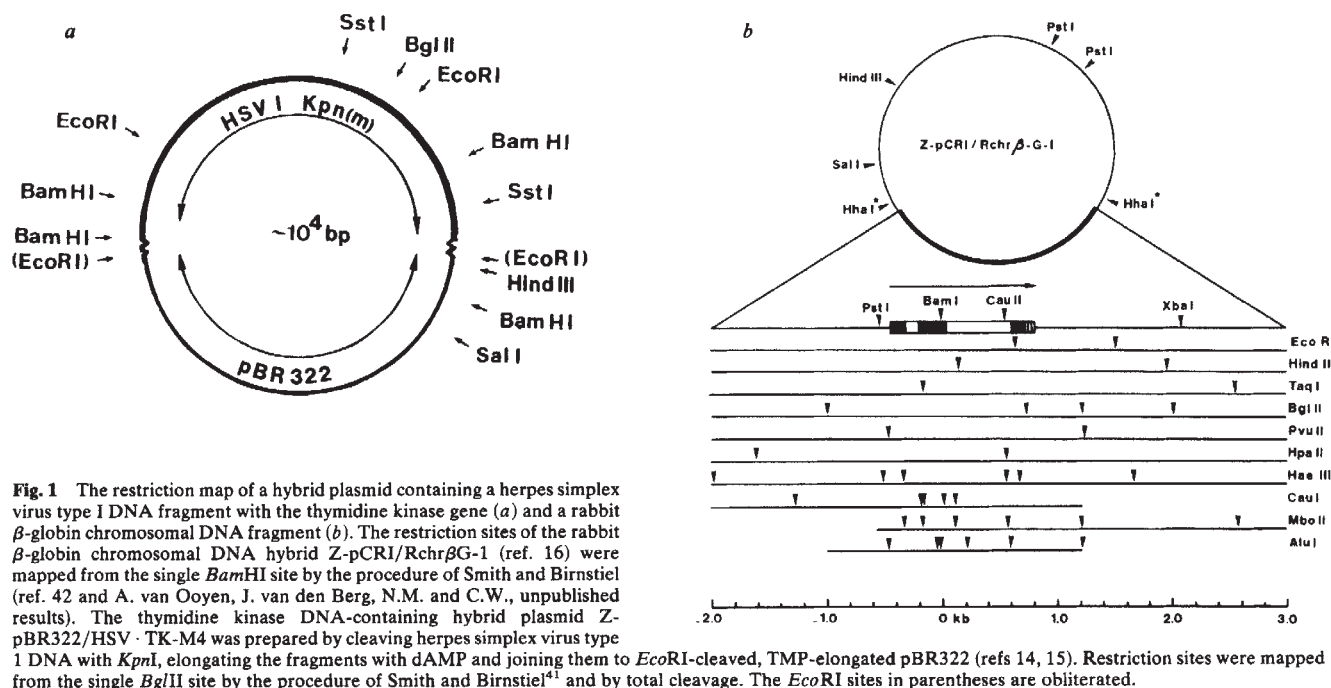


Fig. 1 The restriction map of a hybrid plasmid containing a herpes simplex virus type I DNA fragment with the thymidine kinase gene (a) and a rabbit β -globin chromosomal DNA fragment (b). The restriction sites of the rabbit β -globin chromosomal DNA hybrid Z-pCR1/Rchr β G-1 (ref. 16) were mapped from the single *Bam*HI site by the procedure of Smith and Birnstiel (ref. 42 and A. van Ooyen, J. van den Berg, N.M. and C.W., unpublished results). The thymidine kinase DNA-containing hybrid plasmid Z-pBR322/HSV-TK-M4 was prepared by cleaving herpes simplex virus type 1 DNA with *Kpn*I, elongating the fragments with dAMP and joining them to *Eco*RI-cleaved, TMP-elongated pBR322 (refs 14, 15). Restriction sites were mapped from the single *Bgl*II site by the procedure of Smith and Birnstiel⁴¹ and by total cleavage. The *Eco*RI sites in parentheses are obliterated.

Table 1 Rabbit β -globin DNA and β -globin mRNA content of transformed mouse L cell lines

DNA used for transfection	Thymidine kinase	No. transformants	Cell line	Approximate no. rabbit β -globin DNA copies per cell*	Approximate no. rabbit β -globin RNA transcripts per cell†	
					Expt 1	Expt 2
—	TK-P1	83/2	P1/S-1	0	<20	
			P1/S-2	0	<20	
			M2/S-1	NT	<20	
			M2/S-2	0	<20	
	TK-M4	78/2	M4/S-1	NT	<20	
			M4/S-2	0	<20	
			P1/R-1	0	NT	
			P1/R-2	1	NT	
R β G	TK-P1	71/4	P1/R-3	3	300	600
			P1/R-4	8	300	50
			P1/R-5	1	50	400
			P1/R-6	2	<20	
			P1/R-7	6	<20	
			P1/R-8	1	100	
	TK-M2	59/5	M2/R-1	20	2,000	2,000
			M2/R-2	0.25	NT	
			M2/R-3	1	300	
			M2/R-4	8	<20	
			M2/R-5	0.25	200	
			M2/R-6	0.25	200	
			M2/R-7	3	200	
			M2/R-8	3	<20	
R β G	TK-M4	14/4	M4/R-1	1	150	
			M4/R-3	8	200	
			M4/R-5	0	<20	
			M4/R-6	0.25	NT	
			M4/R-7	1	400	

Thymidine kinase-negative mouse L cells (LMTK⁻) were cultured in Dulbecco's modified Eagle's medium (Flow), 10% calf serum (Eurobio) and 30 μ g ml⁻¹ bromodeoxyuridine (BUdR). Before transfection the cells were cultured for three doublings in the absence of BUdR, then plated at 4×10^5 cells per 9-cm dish 24 h before DNA addition. TK-gene-containing plasmid DNA was prepared in P-2 containment conditions. To prepare concatenated DNA for transfection, 1.25 μ g TK-gene plasmid (Z-pBR322/HSV-TK-P1, -M2 or -M4 (refs 14, 15) and 6.25 μ g Z-pCR1/Rchr β G-1 (ref. 16) were digested with *Sal*I (New England Biolabs), heated for 10 min at 65°C to inactivate the enzyme, and mixed into a final volume of 0.1 ml with 70 mM Tris-HCl (pH 7.2), 10 mM MgCl₂, 10 mM dithiothreitol and 1 mM ATP. Ligation was with T₄ DNA ligase for 3 h at 16°C; no monomeric DNA was detectable by agarose gel electrophoresis. After phenol extraction, ether extraction and ethanol precipitation, the DNA was mixed with 80 μ g salmon sperm DNA and used for transfection of five dishes of LMTK⁻ cells according to the protocol of Wigler *et al.*¹⁷. Control cells were transfected with *Sal*I-cleaved TK-gene plasmid, without ligation. After 19 days in modified HAT¹⁵ medium (15 μ g ml⁻¹ hypoxanthine, 1 μ g ml⁻¹ aminopterin, 5 μ g ml⁻¹ thymidine in Dulbecco's modified Eagle's medium), colonies were picked with cloning rings and grown up for analysis (see Figs 2, 4 and 5).

* The β -globin DNA content of 10–30 μ g of total cell DNA was estimated visually from the autoradiogram of Fig. 2 and additional similar experiments.

† The β -globin RNA content was estimated visually from the autoradiograms of Weaver-Berk-Sharp analyses. Expt 1: the probe used was [5'-³²P]-labelled *Bgl*II-cleaved P β G (data not shown). Expt 2: from the experiment of Fig. 4A. Calculations are explained in the text. NT, not tested.

only cells possessing thymidine kinase activity can survive and grow in a medium containing hypoxanthine, aminopterin and thymidine (HAT medium)¹³, it is possible to select from among 10^6 – 10^7 cells the few tens or hundreds of cells which were transformed by DNA containing the thymidine kinase gene⁹. We have used this system to select mouse cells transformed with cloned rabbit chromosomal β -globin DNA linked to a cloned herpes simplex virus type 1 (HSV1) DNA fragment containing the thymidine kinase (TK) gene. We find that 19 of 21 cell lines selected in HAT medium contain rabbit β -globin DNA, and 12 of 16 such lines synthesise rabbit β -globin-specific RNA which is indistinguishable from natural β -globin mRNA in all aspects tested.

Transformation of TK⁻ mouse cells with rabbit β -globin DNA linked to HSV1-TK DNA

We have prepared hybrid plasmids consisting of pBR322 linked to a fragment of HSV1 DNA containing the TK gene, either a *Bam*HI (Z-pBR322/HSV-TK-P1) or a *Kpn*I fragment (Z-pBR322/HSV-TK-M2 and Z-pBR322/HSV-TK-M4)^{14,15}. Figure 1 shows the restriction maps of the hybrid plasmid Z-pBR322/HSV-TK-M4 (a) and of the hybrid Z-pCRI/Rchr β G-1 (b, abbreviated to R β G), which consists of pCRI linked to a β -globin chromosomal rabbit DNA fragment¹⁶. The plasmids containing β -globin and thymidine kinase DNA were cleaved at their single *Sal*I sites and joined at a molar ratio of 2.5:1 by *T*₄ DNA ligase to form concatenates. TK⁻ L cells were transformed with the concatenates (1.5 μ g total DNA per 8×10^5 cells) using the calcium phosphate method⁸ as described by Wigler *et al.*¹⁷. Cells transformed in parallel with a *Sal*I-cleaved thymidine kinase plasmid alone served as a control. After 19 days in HAT-containing medium 8–47 colonies per dish were recovered (Table 1). Each of 27 colonies was expanded to about 6×10^7 cells, and DNA and RNA were isolated.

Identification of rabbit β -globin DNA in thymidine kinase-transformed mouse L cells

The DNA from the thymidine kinase-transformed cell lines was cleaved with *Eco*RI and *Pst*I, denatured, subjected to agarose gel electrophoresis and transferred to nitrocellulose filters by the procedure of Southern¹⁸. As reference, the chromosomal β -globin DNA hybrid R β G, cleaved with the same enzymes, was run in parallel. The filter was hybridised with a ³²P-labelled, cloned rabbit β -globin cDNA probe devoid of plasmid sequences¹⁹. As shown in the restriction map of Fig. 1, cleavage of rabbit chromosomal DNA by *Pst*I and *Eco*RI is expected to yield two β -globin-specific fragments of 1,200 and 900 base pairs, respectively. The autoradiogram (Fig. 2) shows that the four DNA preparations from cells transformed with the rabbit β -globin-TK DNA concatenates had two bands with the same mobilities and the same relative intensities as the 900- and 1,200-base pair bands derived from the β -globin chromosomal plasmid. Altogether, 19 of 21 TK-positive cell lines contained β -globin DNA by this analysis. The control, DNA from cells transformed with a TK plasmid only, did not show these characteristic bands. From the intensities of the bands of *Eco*RI- and *Pst*I-cleaved cell DNA relative to those of known amounts of similarly treated cloned chromosomal DNA run in parallel, we estimate visually that the cell lines P1/R-3, P1/R-4, P1/R-5 and M2/R-1 contain about 3, 8, 1 and 20 copies of rabbit β -globin DNA per cell (Table 1).

Identification and characterisation of rabbit β -globin-specific transcripts from transformed mouse L cells

In vivo transcription of the extraneous rabbit β -globin gene was monitored by a modification of the Berk-Sharp procedure²⁰

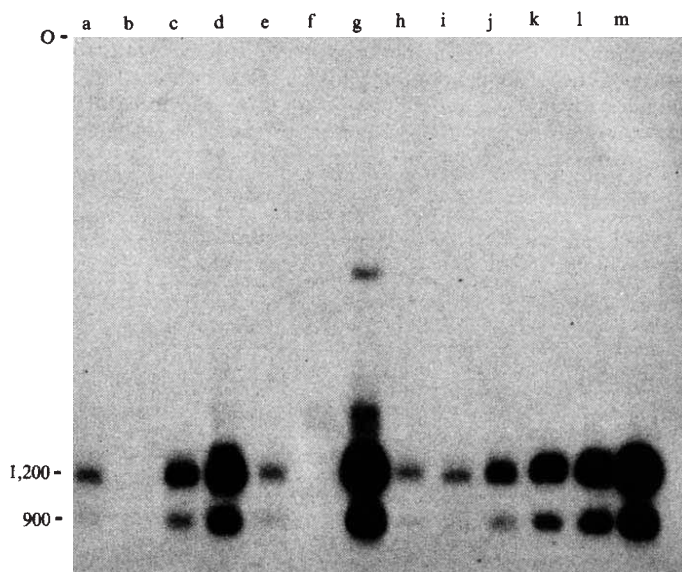


Fig. 2 Detection of chromosomal rabbit β -globin sequences in DNA of transformed mouse L cell lines. Mouse L cell lines transformed with the rabbit β -globin hybrid DNA linked to a thymidine kinase plasmid, or with a thymidine kinase plasmid only, were obtained as described in Table 1 legend. 1.5×10^7 cells were lysed with 1.1% SDS in 0.15 M NaCl and 0.1 M EDTA (pH 8), extracted with CHCl_3 and isoamyl alcohol (24:1), and the DNA spooled during addition of ethanol to 66%. After treatment with pancreatic RNase, 20 $\mu\text{g ml}^{-1}$ in 10 mM NaCl, 0.5 mM EDTA and 5 mM Tris-HCl (pH 7.5) for 8 h at 37°C, overnight incubation at 37°C with 100 $\mu\text{g ml}^{-1}$ pronase in 100 mM NaCl, 5 mM EDTA and 50 mM Tris-HCl (pH 7.5) and phenol extraction, the DNA was collected by spooling as above. About 300–600 μg of DNA were recovered. Cell DNA (10 μg) was digested with *Pst*I (MRC, Porton) and *Eco*RI (MRC, Porton), denatured and subjected to electrophoresis on a 1.4% agarose gel as described by Jeffreys and Flavell⁴³. The DNA was transferred to a 15×17 cm Millipore membrane¹⁸ and hybridised to a ³²P-labelled rabbit β -globin probe (specific activity, 8×10^7 c.p.m. per μg ; about 325 ng in 25 ml) as described⁴³. The probe was obtained as follows. The rabbit β -globin cDNA-containing hybrid plasmid P β G⁴⁴ was cleaved with nuclease S₁ in formamide⁴⁵, joined to *Hind*III linkers⁴⁶ and recloned in the *Hind*III site of pBR322 (ref. 19). A hybrid plasmid containing the β -globin sequence present in P β G was identified by nucleotide sequence analysis (Z-pBR322-H3/Rc β G-4.13) and prepared in quantity. After cleaving with *Hind*III, the β -globin-specific fragment was purified by agarose gel electrophoresis and nick-translated⁴⁷ using [α -³²P]-labelled dATP and dCTP (400 Ci mmol⁻¹ each; NEN, Dreieich). Autoradiography of the filter was for 30 days. Lanes b and f, DNA from cell lines P1/S-2 and M2/S-2, transformed only with a *Sal*I-cleaved TK-gene plasmid. Lanes c, d, e and g, DNA from cell lines P1/R-3, P1/R-4, P1/R-5 and M2/R-1, transformed with the rabbit β -globin containing hybrid R β G, linked to a TK-gene plasmid. Lanes a and h–m contain as reference R β G digested with *Pst*I and *Eco*RI: 1.6, 1.6, 1.6, 3.2, 6.4, 12.8 and 32 μg of DNA hybridisable to the probe (1.8 μg is the equivalent of one β -globin gene). Lanes a, h and j–m also contain 10 μg of DNA from cell line P1/S-2 digested with *Pst*I and *Eco*RI as carrier. The mobility of the 900- and 1,200-base pair bands is about 1% less in the presence of 10 μg total DNA per slot (compare lanes h and i) than in its absence. O, origin. Ordinate in base pairs.

developed by Weaver *et al.*²¹, as exemplified in Fig. 3. The hybrid plasmid R β G contains a *Bgl*II site immediately following the 3'-end of the β -globin coding sequence. The hybrid was cleaved with *Bgl*II and labelled with ³²P at the 5'-termini. After digestion with *Pst*I, the *Pst*I-*Bgl*II (1,291/1,299) fragment, labelled at the *Bgl*II site (on the (–)strand), was isolated. The labelled DNA was denatured, hybridised with the RNA sample in 80% formamide, treated with nuclease S₁ and analysed by polyacrylamide gel electrophoresis in 7 M urea. Only if the labelled 5'-terminus of the DNA is protected by RNA complementary to it is a radioactive fragment recovered. As the probe is labelled on the (–)strand, only (+)strand RNA is detected. Mature rabbit β -globin mRNA protects a fragment about 134 nucleotides long (from the *Bgl*II site to the 3'-end of the large intervening sequence (intron)), whereas a precursor containing both introns would protect about 1,200 nucleotides and a precursor containing only the large intron would protect 929 nucleotides.

The probe discriminates between mouse and rabbit globin mRNA because the rabbit mRNA has a *Bgl*II recognition

Fig. 3 Application of the Weaver-Berk-Sharp method to distinguish mature and precursor rabbit β -globin mRNA in the possible presence of mouse β -globin mRNA. The procedure is a modification of the method of Berk and Sharp²⁰. The probe is a DNA restriction fragment 32 P-labelled at one 5'-terminus (rather than being uniformly labelled). The denatured DNA is hybridised to the RNA sample in 80% formamide, digested with S_1 nuclease, denatured and analysed by polyacrylamide gel electrophoresis in 7 M urea. Only if the 5'-terminus of the probe was hybridised to RNA is a labelled DNA fragment recovered. The Pst - Bgl III ((+)-strand 1,291/(-)-strand 1,299 bases) fragment derived from the rabbit chromosomal β -globin plasmid (see Fig. 1), labelled at the Bgl III site, is diagnostic for (+)-strand β -globin sequences and allows the distinction between rabbit precursor RNA, rabbit mature RNA and mouse RNA. E, exon; I_s and I_L , small and large intron, respectively. PN-Kinase, polynucleotide kinase.

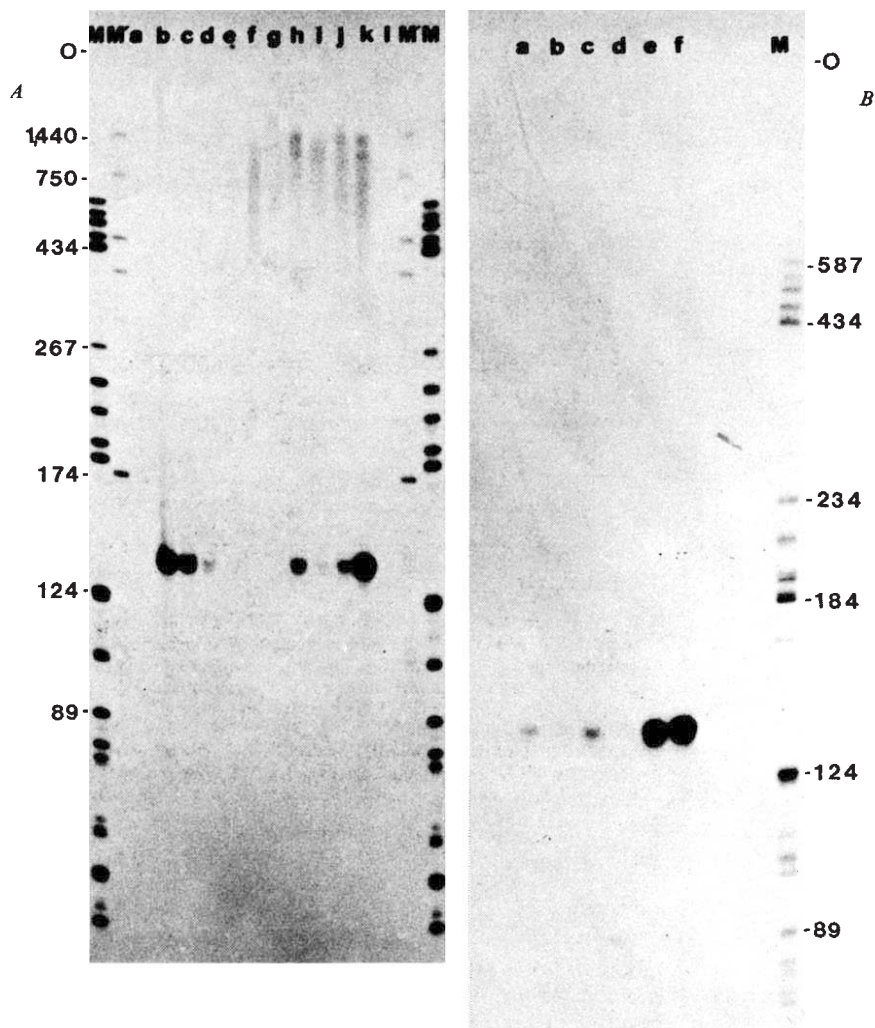
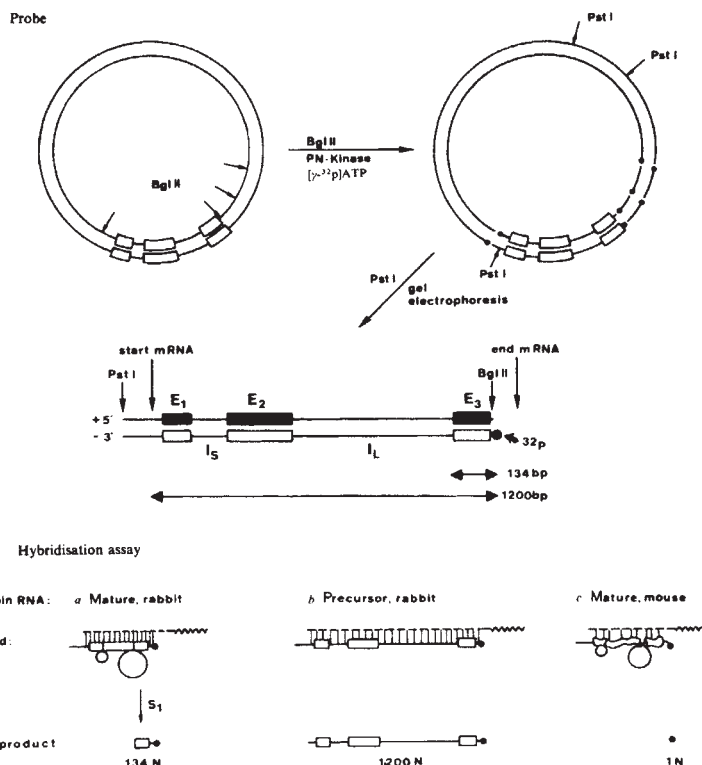


Fig. 4 Detection and characterisation of rabbit β -globin RNA in transformed mouse L cells by the Weaver-Berk-Sharp method. Total cell RNA was prepared from about 10^8 cells as described elsewhere²⁹. The yield was about 1.6 mg per 10^8 cells. The 32 P-labelled probe (described in Fig. 3) was prepared by cleaving 20 μ g Z-pCR1/Rchr β G-1 with Bgl III (New England Biolabs), treating with bacterial alkaline phosphatase (BAPF, Worthington) and labelling with polynucleotide kinase (P-L Biochemicals) and [γ - 32 P]ATP (specific activity 3,300 Ci mmol⁻¹, NEN) as described elsewhere²⁸. After purification on a Sephadex G-50 column, the DNA was cleaved with Pst I (MRC, Porton), and the labelled 1,291–1,299-base pair fragment isolated after electrophoresis through a 1.5% agarose gel and passed through a Chelex column. About 25 ng of probe (13,000 c.p.m.) were denatured by heating in 80% formamide for 15 min at 100°C and mixed with 50 μ g RNA. Hybridisation was carried out in 10 μ l of 0.4 M NaCl, 0.04 M PIPES (pH 6.4), 0.001 M EDTA and 80% formamide, at 57°C for 16 h under paraffin oil. The sample was diluted with 100 μ l 0.25 M NaCl, 0.03 M sodium acetate (pH 4.5) and 0.002 M ZnSO₄ and digested with 50 units of S_1 nuclease (purified by the procedure of Wiegand *et al.*⁴⁹). After CHCl₃ extraction to remove the paraffin oil, and precipitation with ethanol, the samples were dissolved in 5 μ l 90% formamide, 0.05% bromophenol blue and 0.05% xylene cyanol FF, heated for 10 min at 100°C, and analysed by electrophoresis through a 0.1 $\times$ 28 $\times$ 40 cm 5% polyacrylamide gel (acrylamide: bis-acrylamide, 30:1) in 0.089 M Tris base, 0.089 M boric acid, 2.5 mM EDTA and 7 M urea. Autoradiography was carried out with use of an Ilford Fast Tungstate intensifier screen at -70°C for 2–5 days. **A**, Unfractionated RNA samples. Lanes a–e, 0, 3, 1, 0.3 and 0.1 ng rabbit globin mRNA ($\alpha + \beta$). Lanes f, g, RNA from cell lines P1/S-1 and P1/S-2, which had been transformed only with a Sa I-cleaved TK-gene plasmid. Lanes h–k, RNA from cell lines P1/R-3, P1/R-4, P1/R-5 and M2/R-1, transformed with the rabbit β -globin DNA-containing hybrid linked to a TK-gene plasmid. Lane l, 50 ng mouse globin mRNA. Lanes M, M', [γ - 32 P]-labelled fragments of plasmid pBR322 as size markers⁵⁰. **B**, Poly(A)⁺ and poly(A)⁻ fractions of RNA samples. RNA (50 μ g) from cell line M2/R-1 or rabbit globin mRNA (3 ng) was passed through a

0.1 ml oligo(dT) cellulose (type 7, P-L Biochemicals) column in 0.2 M NaCl, 0.01 M EDTA, 0.1% SDS and 0.1 M Tris-HCl (pH 7.5)⁵¹. The column was washed with the same buffer and eluted with H₂O. The RNA in the flow-through and wash (poly(A)⁻) and in the H₂O eluate (poly(A)⁺) was precipitated with ethanol. Lanes a, b, poly(A)⁺ and poly(A)⁻ fractions of M2/R-1 RNA; lanes c, d, poly(A)⁺ and poly(A)⁻ fractions of rabbit globin mRNA. Lanes e, f, 20 and 40 μ g unfractionated rabbit bone marrow RNA. Lane M, as above; O, origin. Ordinate in base pairs.

sequence²² whereas mouse mRNA does not (ref. 23 and N.M., unpublished results), and therefore does not protect the labelled 5'-terminus of the probe. Figure 4A shows that as little as 0.1 ng rabbit β -globin mRNA (lane e) gave the expected 134-nucleotide fragment whereas 50 ng mouse globin mRNA (lane l) gave no such band. The RNA from transformed cell lines P1/R-3, P1/R-4, P1/R-5 and M2/R-1 (lanes h-k) yielded as major product the 134-nucleotide fragment. Thus, the majority of globin-specific transcripts are partly or entirely devoid of the large intron. Some weak bands corresponding to fragments of 750–1,200 nucleotides may be due to unprocessed or incompletely processed precursor molecules. RNA from cells transformed with only a thymidine kinase plasmid (lines P1/S-1 and P1/S-2, lanes f and g) gave no detectable bands. From the amount of cell RNA added to the assay (50 μ g), the amount of RNA per cell (about 25 pg)²⁴ and the fact that the intensity of the 134-nucleotide band is similar to that given by 3 ng of total rabbit globin mRNA (about half of which is β -specific), we estimate that cell line M2/R-1 contains about 2,000 rabbit β -globin RNA molecules per cell. By the same criteria, P1/R-3, P1/R-4 and P1/R-5 contain about 600, 50 and 400 β -globin transcripts per cell. As relative band intensities were estimated visually, these values as well as those in Table 1 are only approximate. Altogether, 12 of 16 globin DNA-containing clones had β -globin-specific transcripts (see Table 1).

To determine whether the small intron was present, RNA was hybridised to R β G DNA which had been digested with *Bam*HI, labelled at the 5'-termini and cleaved again with *Bgl*II. After *S*₁ cleavage and denaturation, polyacrylamide gel electrophoresis revealed a 212-nucleotide fragment, in the case of both authentic rabbit β -globin mRNA and RNA from samples P1/R-3, P1/R-4, P1/R-5 and M2/R-1 (Fig. 5). This shows that the small intron was not present in the transcripts, because otherwise a 480-nucleotide fragment would have appeared.

The 5'-terminus of the β -globin-specific transcripts was mapped by the same procedure as before, using as probe a restriction fragment extending from the *Mbo*II cleavage site at position 129/128 to the *Pst*I site at position -95/-99, [³²P]-labelled at the *Mbo*II-cleaved end. As shown in Fig. 6, natural rabbit β -globin mRNA gave rise to a labelled fragment 128 nucleotides long; RNA from the cell line M2/R-1 gave rise to a strong and P1/R-3 to a weaker band in the same position as the β -globin mRNA; in the case of RNA from cell lines P1/R-4 and P1/R-5, only very weak bands were discernible on the original films (but not on the photograph), perhaps because of the relatively low content of β -globin-specific RNA in these cell lines (Table 1). This experiment showed that the only detectable 5'-terminus of the β -globin transcripts mapped in the same position as that of authentic β -globin mRNA; however, we cannot exclude the possibility that a certain proportion of molecules may have a variety of 5'-termini which escaped detection because of a broad distribution of the resulting bands.

The presence of poly(A) on the β -globin-specific transcripts from transformed L cells was demonstrated by fractionating RNA from M2/R-1 on an oligo(dT) column and assaying separately the flow-through fraction [poly(A)⁻] and the fraction retained at high salt [poly(A)⁺], using as probe the *Pst*I-*Bgl*II (1,291/1,299) fragment 5'-labelled at the *Bgl*II terminus. About 80% of the β -globin transcript was detected in the poly(A)⁺ fraction (Fig. 4B).

The mobility of the rabbit β -globin-specific RNA present in the cell line M2/R-1 was compared with that of natural rabbit globin mRNA using the 'Northern transfer' procedure of Alwine *et al.*²⁵ The RNAs were subjected to electrophoresis on an agarose gel in denaturing conditions, transferred to diazobenzyloxymethyl paper and hybridised to ³²P-labelled rabbit β -globin cDNA¹⁹. As shown in Fig. 7, poly(A)⁺ RNA derived from 150 μ g of M2/R-1 RNA gave an autoradiographic band with the same mobility and the same intensity as about 8 ng of authentic rabbit globin mRNA; no band was found with the poly(A)⁺ RNA from 150 μ g of RNA from cells transformed with TK DNA only.

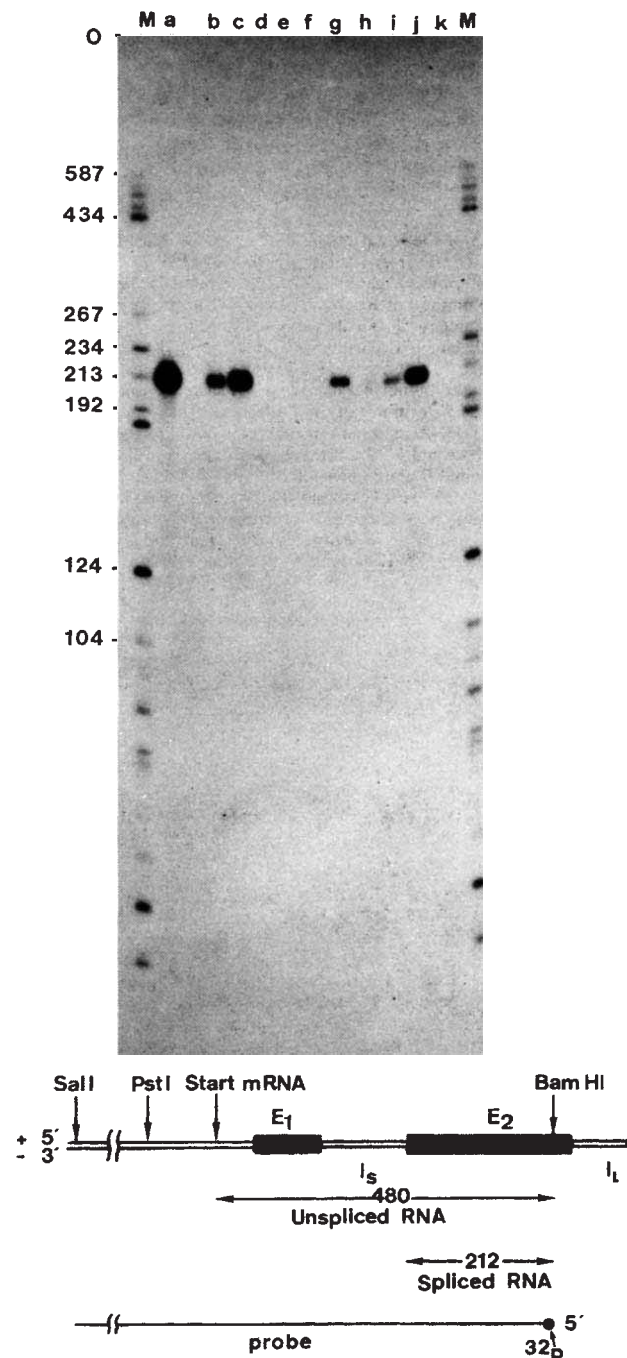


Fig. 5 Assay for the presence of the small intron in rabbit β -globin transcripts from transformed mouse cells. The RNA preparations are those described in Fig. 4 legend. The probe was prepared by digesting Z-pCRI/Rchr β G-1 with *Bam*HI, labelling the 5'-terminus with ³²P-phosphate (specific activity 2,100 Ci mmole⁻¹) and cleaving with *Bgl*II. Lanes a-c, 3, 0.3 and 1.1 ng rabbit globin RNA ($\alpha + \beta$). Lane d, 50 ng mouse globin RNA. Lanes e, f, RNA from cell lines P1/S-1 and P1/S-2, transformed only with a *Sal*I-cleaved TK-gene plasmid. Lanes g-j, RNA from cell lines P1/R-3, P1/R-4, P1/R-5 and M2/R-1, transformed with the rabbit β -globin DNA-containing hybrid linked to a TK-gene plasmid. Lane k, no RNA. M, Size marker: pBR322 digested with *Bsp*I and [³²P]-labelled.

Finally, we determined whether transformed L cells produce mouse β -globin mRNA, in particular when they are synthesising rabbit β -globin mRNA. We again used the Weaver-Berk-Sharp assay, with the [³²P]-labelled 123-nucleotide *Bsp*-*Bsp* (-) strand fragment of cloned mouse β -globin cDNA²⁶ as probe. This (-)strand fragment, which corresponds to the mouse β -globin mRNA segment extending from nucleotide 222 to 344, was purified by hybridising it to mouse β -globin mRNA,

isolating the hybrid on an oligo(dT) column and recovering the DNA after alkaline hydrolysis. The assay was carried out as described above; 0.1 ng of mouse β -globin mRNA gave a strong positive signal whereas 10 ng rabbit globin mRNA gave no band, as a sequence complementary to the ^{32}P -labelled terminus of the probe is not present on the rabbit β -globin mRNA. RNA (50 μg total) from L cell lines transformed with TK DNA only (P1/S-1, P1/S-2) or with rabbit β -globin-TK DNA concatenates (P1/R-3, P1/R-4, P1/R-5, M2/R-1) gave no detectable signal (data not shown). From this experiment we estimate that the transformed L cell lines tested, in particular that with 2,000 molecules rabbit β -globin mRNA per cell (M2/R-1), contained less than 50 strands of mouse β -globin mRNA per cell.

Discussion

Concatenates containing a high ratio of β -globin DNA to TK DNA proved to be a convenient device for introducing into mouse L cells multiple β -globin gene copies together with a selective marker which permitted the isolation of transformed cells. Previous experience²⁷ suggests that the exogenous DNA is largely integrated into host cell sequences, but this point requires further investigation.

Most of the cell lines transformed with rabbit β -globin DNA produced rabbit β -globin-specific RNA. It is striking that the cell line M2/R-1, which contained the highest number of gene copies per cell (about 20), also contained the highest number of RNA molecules (2,000 per cell); however, this correlation did not hold throughout.

The rabbit β -globin-specific RNA of line M2/R-1 was examined in closest detail and was found to be indistinguishable from natural mRNA with regard to its electrophoretic mobility, its 5'-terminus, the absence of the large and small introns and the presence of a 3'-terminal poly(A) tail. The β -globin RNAs of the other cell lines, when tested for one or the other of the criteria, had similar properties.

Synthesis of β -globin mRNA in the mouse occurs through a short-lived 15S precursor²⁸⁻³² which represents a complete transcript of the gene, including the introns³³⁻³⁵. The same is apparently true for the rabbit β -globin mRNA³⁶. It is clear from our results that the splicing enzyme(s) of the mouse cell is capable of acting effectively on the rabbit β -globin transcript. A comparison of the nucleotide sequences of the rabbit and mouse β -globin genes had shown that although the internal sequences of the homologous introns were very different^{16,53}, the homologous intron-exon junction regions were very similar. The fact that the DNA of SV40, a monkey virus, when injected into *Xenopus* oocytes, leads to the formation of apparently normal SV40 proteins shows that the splicing systems of even widely divergent species are compatible³⁷. However, yeast seems to be incapable of splicing the rabbit β -globin transcript³⁸.

Ziff and Evans³⁹ have shown that in the case of the adenovirus late mRNA, the cap-carrying 5'-terminus represents the initiation site of transcription. In the case of mouse β -globin RNA the 15S precursor and the mature mRNA have the same 5'-terminal sequence²¹ and the same cap structures²⁶. If, as we believe, the 15S β -globin precursor is the primary transcript (but for a contrary view, see ref. 31), we may conclude that the rabbit β -globin RNA produced in transformed mouse cells (at least in cell lines P1/R-3 and M2/R-1) originates at the true promoter. We are not certain whether other 5'-termini occur at lower levels, and we cannot yet exclude the possibility that the transcript is initiated upstream at a plasmid or a host promoter, and that the correct, capped 5'-terminus arises by processing. In this connection it is interesting that β -globin transcripts generated in yeast transformed with chromosomal β -globin DNA have 5'-termini which map downstream from the correct 5'-terminus, and 3'-termini mapping within the large intron³⁸.

No mouse β -globin mRNA was found in TK- or β -globin DNA-transformed mouse L cells used in our experiments (<50 molecules per cell), even when they contained 2,000 rabbit

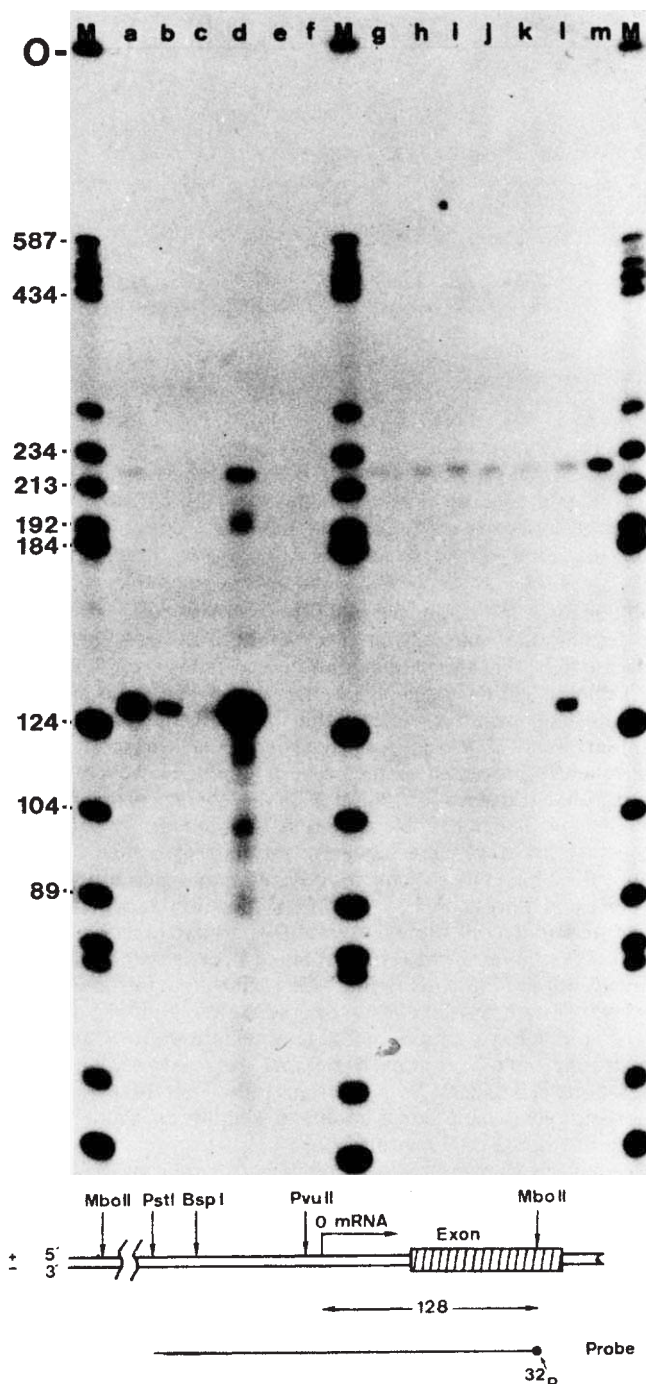


Fig. 6 Mapping of the 5'-terminus of rabbit β -globin-specific transcripts from transformed mouse L cells. The RNA preparations are those described in Fig. 4 legend. The probe for mapping the 5'-end was prepared as follows. Plasmid Z-pCRI/Rchr β G-1 was digested with *Hha*I and the largest fragment isolated by centrifugation through a 5–23% sucrose density gradient in a Spinco SW41 rotor, at 30,000 r.p.m. for 12 h at 15 °C. The 6.4-kilobase *Hha*I fragment was cleaved with *Mbo*II (New England Biolabs), and labelled with polynucleotide kinase and [γ - ^{32}P]ATP (specific activity, 3,200 Ci mmol⁻¹). After cleavage with *Pst*I, the 223-base pair fragment was isolated by electrophoresis through a 1.6% agarose gel. Lanes a–c, 3, 1 and 0.3 ng rabbit globin mRNA. Lane d, 20 μg rabbit bone marrow RNA. Lane e, no RNA. Lane f, 50 ng mouse globin mRNA. Lanes g, h, RNA from cell lines P1/S-1 and P1/S-2, transformed only with a *Sa*I-cleaved TK-gene plasmid. Lanes i–l, RNA from cell lines P1/R-3, P1/R-4, P1/R-5 and M2/R-1, transformed with the rabbit β -globin DNA-containing hybrid linked to a TK-gene plasmid. Lane m, DNA probe not digested with *S*₁ nuclease. M, Size marker: pBR322 digested with *Bsp*I and [γ - ^{32}P]ATP-labelled.

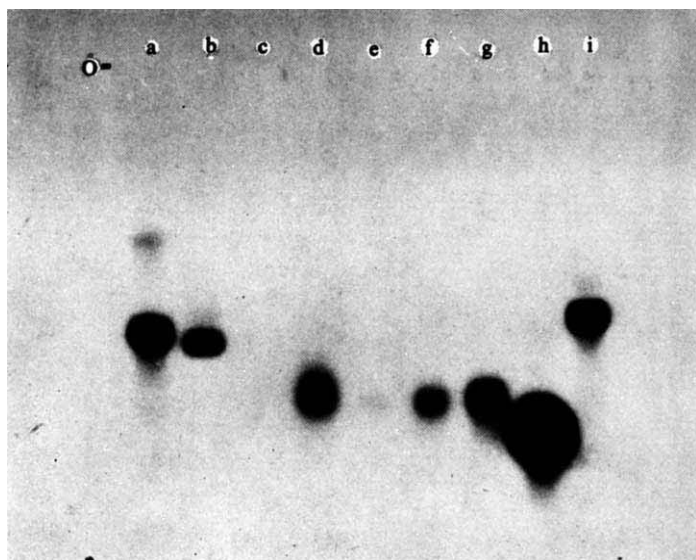


Fig. 7 The electrophoretic mobility of rabbit β -globin transcripts from transformed mouse L cells. Poly(A)⁺ RNA was prepared as described in Fig. 4 legend. β -Globin mRNA and denatured restriction fragments of rabbit β -globin-specific DNA were used as size markers. All samples (10 μ l) were mixed with 40 μ l of a mixture consisting of 100 μ l 0.1 M sodium phosphate buffer (pH 7), 200 μ l glyoxal (Merck, Darmstadt; passed three times through BioRad AG-501-X8 CD) and 500 μ l dimethyl sulphoxide (Fluka), and heated at 50°C for 60 min²². Electrophoresis was through a 14 $\times$ 10 $\times$ 0.5 cm 1.5% agarose gel in 10 mM sodium phosphate (pH 7). The nucleic acid was transferred to diazobenzyloxymethyl paper as described by Alwine *et al.*²⁵ and hybridised with the ³²P-labelled β -globin DNA probe (1.6 $\times$ 10⁸ c.p.m. per μ g) described in Fig. 2 legend. (a) R β G DNA digested with PstI and BglII (1,299 base pairs). (b) R β G DNA digested with PvuII and BglII (1,209 base pairs). (c) Poly(A)⁺ RNA from 150 μ g of P1/S-1 cells (transformed with TK DNA only). (d) Poly(A)⁺ RNA from 150 μ g of M2/R-1 cells (transformed with rabbit β -globin DNA and TK DNA). (e-g) Rabbit β -globin mRNA 1, 4 and 8 ng, respectively. (h) Cloned rabbit β -globin cDNA (HindIII-cleaved Z-pBR322 · H3/Rc β G-4.13, see Fig. 2 legend; ~540 base pairs). (i) R β G cleaved with PstI and BglII (1,299 base pairs). All samples contained 20 μ g yeast RNA as carrier. O, Origin.

globin mRNA molecules per cell. The simplest explanation for the finding that the extraneous rabbit β -globin genes are expressed but that the endogenous mouse genes are not is that the rabbit β -globin genes are being transcribed from a non-physiological promoter (either within or outside the rabbit DNA segment), and that the resulting oversized transcripts are subsequently processed to the correct length. However, if we assume that transcription initiates at the correct promoter, it would seem that the rabbit promoter is at most only partially subject to whatever mechanism controls expression of the mouse β -globin genes: if control is *cis* effective the rabbit gene may escape repression because of the position at which it is integrated or, if a diffusible repressor is operative (*trans* control) it may fail to interact with the rabbit control sequence. Alternatively, both rabbit and mouse β -globin RNAs are produced at low levels, but the rabbit RNA is degraded slowly and the mouse RNA rapidly. Explanations invoking gene dosage effects such as the titrating-out of a putative repressor⁴⁰ are less likely, because at least one cell line, P1/R-5, contains only one rabbit β -globin gene and yet accumulates rabbit β -globin mRNA but no detectable mouse β -globin mRNA.

Berg and coworkers¹² have replaced a segment of the SV40 late region by a cloned cDNA copy of rabbit β -globin mRNA.

Monkey cells infected by such hybrid DNA underwent a lytic cycle, during which the DNA was greatly amplified. Hybrid mRNA molecules containing the β -globin sequence were transcribed from an SV40 promoter, leading to the accumulation of >10⁴ hybrid mRNA molecules per cell (P. Berg, personal communication); β -globin was efficiently and accurately translated. We have not yet analysed the proteins produced in our transformed mouse cells, but it seems reasonable to assume that β -globin synthesis, if it occurs, will bear some relationship to the β -globin mRNA content of the cell, and will thus be substantially lower than in the lytic SV40 hybrid system. Thus, thymidine kinase-linked transformation will probably be useful for the study of initiation of transcription and splicing and the SV40 hybrid system for the investigation of translation.

After this manuscript was submitted Wigler *et al.*⁴¹ reported co-transformation of mouse L cells with rabbit β -globin DNA and HSVI-TK DNA.

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letters

Absorption spectrum of the accretion column in VV Puppis

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VV Puppis is an AM Herculis type binary system exhibiting variable circular and linear polarisation¹. In its 'normal' state, the optical light curve shows a slow rise to maximum ($m_{\max} \sim 15-16$) and a rapid drop to minimum ($m_{\min} \sim 17.5-18$) where it remains for $\sim 60\%$ of its 100-min period²⁻⁴. However, the system has also been observed in faint inactive states⁵ ($m_{\min} \sim m_{\max} \sim 15$). Only a few spectroscopic observations of VV Pup have been published and these show a continuous spectrum with emission lines of hydrogen and helium superimposed. We present here spectrophotometric observations obtained on 2 February 1979. Broad absorption features are seen for the first time in this object in the optical spectrum during the bright phases with high circular polarisation. We suggest that these features be identified with the 6th, 7th and 8th harmonics of cyclotron absorption in a magnetic field of $\sim 3 \times 10^7$ G.

The observations were obtained using the image dissector scanner⁸ modified as a polarimeter at the $f/15$ Cassegrain focus of the 4-m Anglo-Australian telescope. Full details of the scanner-polarimeter system which is similar to that described by Nordsieck⁹ will be given elsewhere.

We obtained 12 scans of VV Pup from 11.48 UT to 14.00 UT with an effective time resolution of 8 min. The spectra cover the wavelength region $\lambda\lambda 3,500-7,000$ Å and the spectral resolution is ~ 10 Å. The visual V magnitude calculated from each reduced scan is plotted in Fig. 1 against the phase of VV Pup computed according to Walker's² ephemeris. The absolute energy distributions for the bright and faint phases are displayed in Fig. 2. The computed mean ($\lambda\lambda 4,000-6,000$ Å) value of circular polarisation during the bright phase at 11.58 UT is $\sim +4.0\%$, and during the bright phase at 13.40 UT is $\sim +6.0\%$.

On the basis of the shape of the optical light curve, (Fig. 1) our observations apparently correspond to an epoch in which VV Pup was in its 'normal' state. However, two important differences should be noted. First, the phase of eclipse ingress differs by $\Delta\phi \sim 0.2$ relative to Walker's ephemeris. This drift in phase is large compared to the maximum drift recorded previously and it could be the result of the period of enhanced mass transfer in March 1978, or a similar event which has occurred more recently. Second, the spectra obtained during the bright phases show broad absorption features covering the spectral regions $\lambda\lambda 4,100-4,600$, $4,600-5,400$, $5,400-6,200$, which have not been previously observed.

Variable circular and linear polarisation observed in VV Pup supports a model in which the primary is a strongly magnetic white dwarf rotating synchronously with the binary period^{10,11}. In suitable conditions the material from the late type secondary will be channelled towards the magnetic poles where in general a shock ($T \sim 10^8$ K) will form^{12,13}. In this model the variable optical radiation originates from a small hot spot at one of the magnetic poles of the white dwarf. The bright epochs with

$m_{\min} \sim m_{\max} \sim 15$ correspond to a situation when the second pole also is active. The faint epochs with $m_{\min} \sim m_{\max} \sim 18$ occur when both poles are inactive.

In the framework of the above model our observations with $m_{\min} \sim 17.8$ and $m_{\max} \sim 15.7-16.5$ (see Fig. 1) correspond to an epoch when only the first pole was active. The observed sign of circular polarisation and lack of circular polarisation during minimum phase are consistent with this hypothesis. The absorption features, which are seen only during the bright phases, are probably associated with the gas at the base of the accretion column near the active magnetic pole. From brightness temperature considerations alone, a Zeeman interpretation due to hydrogen at fields $\geq 10^8$ G seems unlikely. An attractive possibility is that these features are cyclotron absorption harmonics arising from the accretion column which is believed to dominate the optical radiation.

The wavelength of the m th harmonic for cyclotron absorption by electrons in a magnetic field B (G) is given by $\lambda_m = 1/m (10^8/B)$ 10,710 Å. The observed features can thus be identified with the 6th (5,800 Å), 7th (4,977 Å) and 8th (4,380 Å) harmonics with $B = 3.08 \times 10^7$ G. There is also marginal evidence for the fifth (6,960 Å) harmonic. As the observed features are broad, and do not have well defined cores, there is an uncertainty of 1 harmonic in the above identifications with a corresponding uncertainty of $\sim 20\%$ in B . In principle the detection of lower harmonics in the IR will enable a precise determination of B . However, the comparison star is likely to dominate the spectrum of long wavelengths and the detection of low harmonics may not be possible.

An upper limit to the temperature of the radiating column can be obtained by assuming that the features are broadened by the relativistic Doppler effect.

For a mildly relativistic plasma the half width $\Delta\lambda_m$ of the m th harmonic is given by $\Delta\lambda_m/\lambda_m = m^{1/2}(T/(5.9 \times 10^9))$ (ref. 14). Using the observed value of $\Delta\lambda_7/\lambda_7$ 0.06, we find $T = 1.3 \times 10^8$ K. This temperature is consistent with the shock temperature expected for accretion onto a low mass white dwarf¹². For a column of given temperature and thickness there is a critical value m^* of m above which the column becomes optically thin to cyclotron emission, and the intensity of the cyclotron radiation drops rapidly. The observed turnover of the

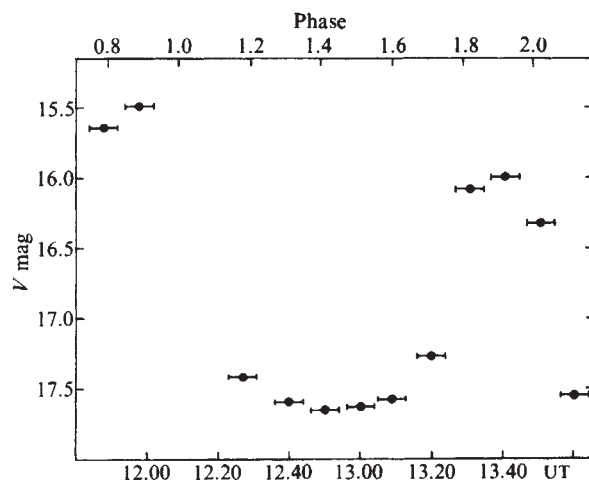


Fig. 1 Light variations for VV Pup during 2 February 1979 from 11.48 UT to 14.00 UT as determined from individual IDS scans with an effective time resolution of 8 min. Phase is given according to Walker's ephemeris.

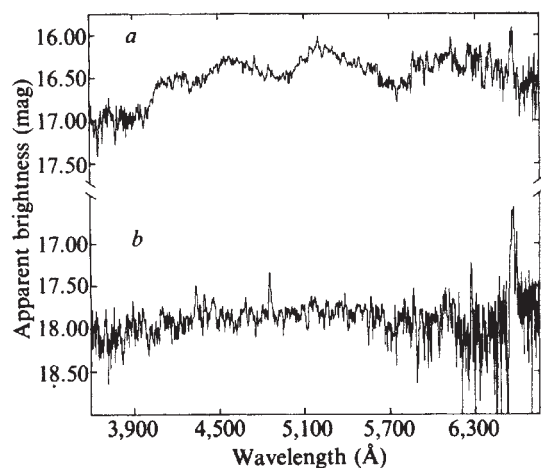


Fig. 2 IDS scans of VV Pup at 10 Å resolution on 2 February 1979 for *a*, the bright (11.48 UT to 11.58 UT) and *b*, the faint (12.27 UT to 13.10 UT) phases.

energy distribution at $\lambda \sim 4,100$ Å could then be interpreted as an optical depth effect.

Because X rays have hitherto not been detected in VV Puppis, there is no direct observational evidence, unlike AM Herculis, for the presence of a shock region. It is therefore possible that the features arise from a cooler region and that magnetic broadening effects are important. However, the eclipse data and luminosity criteria favour a high temperature ($\sim 10^8$ K) emitting region of small dimension^{5,17}.

The absorption features we have observed have not previously been detected in VV Pup. However, the long term variability of the light curve suggests that the height h of the optical column is variable. Values of $10^{-1} R$ and $10^{-2} R$ where R is the radius of the white dwarf, have been reported even at phases when the system had similar maximum brightness^{5,6}. It is not clear how changes in the accretion rate are related to the height h , and width d , of the column. For a dipole field geometry and a cylindrical column at a pole a feature of half width $\Delta\lambda$ will survive magnetic broadening if $h/R \leq 1/3 \Delta\lambda/\lambda$ and $d/R \leq 8/3 \Delta\lambda/\lambda$. For the $\lambda 4,350$ Å feature to be detectable we require $h/R < 0.02$ and $d/R \leq 0.16$. A large increase in h or d could easily result in the obliteration of the absorption features by magnetic broadening thus accounting for their non-detection by previous observers.

These spectra of VV Pup show hitherto unreported absorption features which are most probably high harmonic ($m = 6, 7, 8$) cyclotron absorption in the shock region near the surface of the white dwarf.

If this interpretation is correct, this would be the first determination of the magnetic field in the vicinity of a white dwarf in a close binary system. The analogy with Her X-1 where an X-ray cyclotron feature appropriate to the field of a neutron star has been detected is worth noting. Note also that the field strength deduced for VV Puppis lies in the range of field strengths deduced for isolated white dwarfs by the Zeeman effect^{16,17}.

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Optical counterpart of 2A0311–227

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The X-ray source 2A0311–227 was recently identified independently by Williams¹ and by Griffiths *et al.*². Williams made a tentative identification from an inspection of low dispersion objective prism Schmidt plates taken at Cerro Tololo Inter-American Observatory for the discovery of active galaxies and quasars. The identifying characteristics were strong emission of H and He II. Griffiths *et al.* made the identification from an excellent HEAO-1 position followed by spectroscopic observations. The optical position of the object is $\alpha = 3^{\text{h}} 12^{\text{m}} 00.0^{\text{s}}$, $\delta = -22^{\circ} 46' 48''$ (1950). Figure 1 identifies the X-ray source among the neighbouring stars. It has a mag (variable) of about 15. Immediately after the tentative identification by Williams the 1.3-m telescope at McGraw-Hill Observatory on Kitt Peak was scheduled for spectrophotometric observations of the star in question, and we report the results here. A photon-counting spectral scanner was used. The instrument gives a dispersion of 1.15 Å per channel. Exposures of 5 and 10 min were made that covered the spectral region from 4,000 Å to 5,800 Å. A few scans were taken with lower dispersion and broader spectral coverage. The spectrum shows strong emission features, especially H I, He II $\lambda 4686$ and the C III–N III blend at $\lambda 4640$ – 4650 (ref. 2).

Analysis for radial velocities of the individual spectral scans obtained on the first night showed the star to be a spectroscopic binary of short period. Observations on subsequent nights established a period of 81.04 ± 0.01 min (ref. 1).

The velocity curve for H β is shown in Fig. 2. The phase was computed from HJD $2,443,915.6300 \pm 0.0006 + 0.05628E$. The semiamplitude is 355 km s^{-1} . The velocity curve for He II $\lambda 4686$ is very similar. Any difference in either amplitude or phase is obscured by scatter in the observations. Both H β and He II $\lambda 4686$ show a systemic velocity of $+130 \text{ km s}^{-1}$. However, the projection of the solar motion at this galactic longitude and latitude ($l = 212.9$, $b = -57.4$) is 73 km s^{-1} which gives a galactic radial velocity of $+57 \text{ km s}^{-1}$. The annual proper motion of $+0.147 \pm 0.013''$ and $-0.070 \pm 0.013''$ in right ascension and declination, respectively (W. J. Luyten, personal communication) indicates that the object is no more than a few hundred parsecs away.

The widths of emission lines are approximately 700 km s^{-1} . Their equivalent widths vary with phase. For example, H β changes by a factor of three. However, these equivalent widths must be corrected for variability of the underlying continuum. Preliminary data suggest that variations in the total flux will be small.

Blue and yellow light curves for this object were obtained at Carnegie Southern Observatory (data not shown). There are two maxima and two minima in visual light (5,300 Å); the two minima are of nearly equal depth, but the two maxima differ by 0.4 mag. The total variation is near 1.2 mag, at $V = 13.8$ – 15.0 . A characteristic of the visual light curve seems to be a three-peaked structure at the primary maximum. The separation of the peaks is approximately 6 min and the drop in brightness between peaks is typically 0.3 mag. The central peak, which is also the brightest, occurs at phase 0.775. The maximum is in phase with the maximum velocity of approach. In the blue (4,300 Å) only one maximum is present, which again

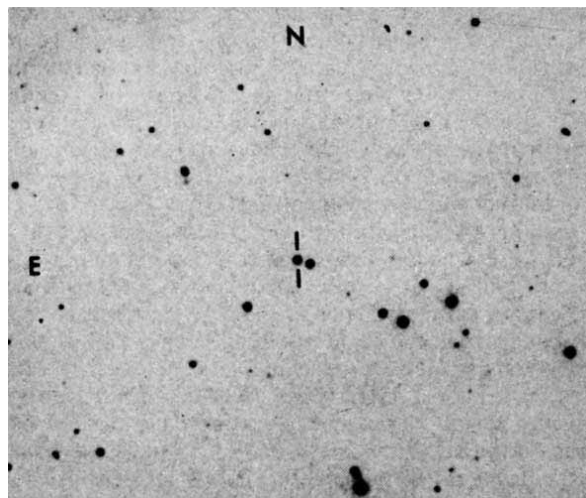


Fig. 1 Identification chart for 2A0311-227.

corresponds in phase to the primary maximum of the visual light curve. The total variation in the blue is about 0.6 mag. During one cycle of the blue light curve a large dip of 0.6 mag was observed during the maximum. This dip, which occurred at phase 0.712, had a width at half-minimum of about 2 min compared with 20-25 min for the maximum on which it occurred. One cycle later the dip was again present, although less pronounced. There is a colour variation, as one would expect from the differences in the blue and yellow light curves, with the star reddest at the two maxima of the yellow light curve.

This system has many characteristics in common with AM Herculis. The period is short (81 compared with 186 min), the spectral characteristics are similar (strong H and He II emission), the light curves for both systems are radically different in blue and yellow radiation³, the two stars show considerable flickering³ and both stars show variable circular and plane polarisation^{4,5}. Each shows a short polarisation pulse that corresponds to maximum light^{4,5}. In this system the polarisation pulse occurs at phase 0.742. Interestingly, this agrees very well with the dip in the visual light curve which precedes the central peak of the primary maximum. Finally, both objects have variable X-ray radiation^{6,7}.

The model for this X-ray source is therefore probably similar to that of AM Her—a white dwarf and a low mass main sequence companion⁸. We suggest a model with a white dwarf of 1.2 solar masses and a companion with 0.2 solar masses. This model follows from the work of Warner who has found that the masses of white dwarfs in cataclysmic variables are typically 1.2 solar masses⁹. He has also found that there is a relationship between the mass ratio and the orbital period. Using this relationship we find a mass of 0.2 solar masses for the secondary. In this model the separation of the two components is $0.7 R_{\odot}$ and the main

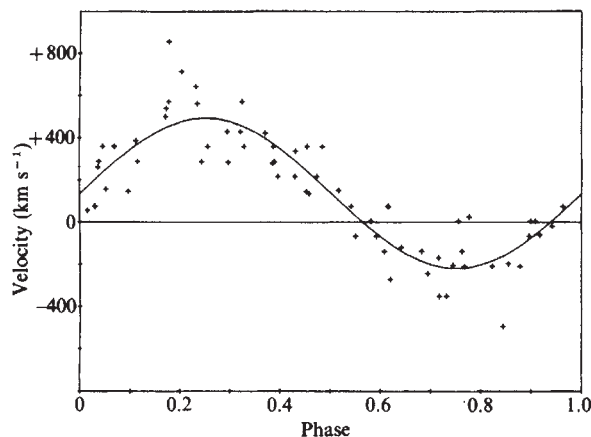


Fig. 2 The H β velocity curve for 2A0311-227.

sequence component will just fill its Roche lobe. The collapsed star is most probably a white dwarf, as circular polarisation is observed⁵. Also, this model is compatible with the estimated distance from the proper motion.

The origin of the emission lines is unclear, but, as in other similar systems, they probably arise from the gas flow from the companion to the white dwarf. Further analysis of observational data may assist in establishing a more critical working model for this unique system.

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Atomic resolution with a 600-kV electron microscope

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The resolution that may be achieved with an electron microscope can be improved by increasing the accelerating voltage, provided that the electrical and mechanical design is of a sufficiently high standard to realise the theoretical performance^{1,2}. Several such microscopes have been constructed in recent years³⁻⁵ and have produced images containing information which can be interpreted directly to a higher resolution than any yet obtained at 100 kV (refs 6, 7). The Cambridge University 600-kV high resolution electron microscope is now yielding high quality micrographs with directly interpretable resolution approaching 0.2 nm. We describe here some of the novel features of this microscope and give some measurements which establish its electron-optical performance. We also show some examples of recent observations which demonstrate the benefits of its atomic resolving power. A more detailed description of the instrument and more references are given elsewhere^{2,8}.

The electron gun incorporates a lanthanum hexaboride thermionic cathode, in the indirectly-heated rod configuration^{9,10}. For operation with a brightness 10 times greater than is normally obtained from a tungsten filament the cathode lifetime is regularly more than 100 h. Such brightness allows observation of images on the fluorescent viewing screen at electron-optical magnifications $> \times 500,000$, and hence permits direct and accurate correction of astigmatism and choice of objective lens defocus without recourse to lengthy trial and error procedures (compare Krivanek *et al.*¹¹). Fringes of 0.27 nm spacing from a

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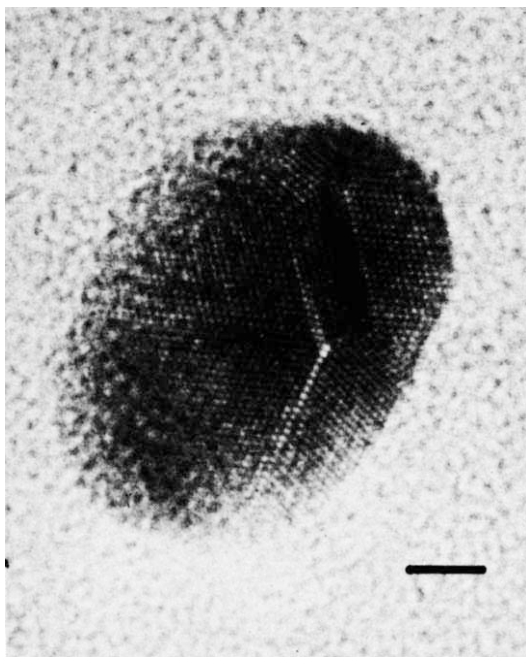


Fig. 1 Micrograph of a small gold particle, supported on an amorphous carbon film, recorded at 575 kV at the optimum defocus. Analysis of the detail in the carbon film defines the imaging conditions and indicates that structure within the particle can be interpreted directly as showing projections of rows of gold atoms separated by 0.235 nm, viewed end-on. Scale bar, 2 nm.

tungsten trioxide specimen have been observed on the fluorescent screen.

The objective lens of the microscope is a symmetrical single-field condenser-objective^{12,13}, with the specimen located close to the centre of the 14-mm polepiece gap. The calculated spherical aberration coefficient for this lens is 3.3 mm, so that for an accelerating voltage of 600 kV the contrast transfer function of the microscope should have a first zero crossing of 0.20 nm at the optimum defocus. This value provides a useful measure of the information which can be interpreted directly from a single micrograph, although details of finer spacing may be observable at this, and other, defocus values. To ensure that this performance is not degraded by electrical instabilities, highly stable lens current supplies have been developed and the 600-kV generator¹⁴ has been modified to reduce ripple and drift.

The entire electron-optical assembly of the microscope, about 5.5 m high and weighing about 7 tonnes, is suspended from three compressed-air vibration isolation units mounted on the walls of the microscope room. Ambient vibrations have been shown to be attenuated by a factor of the order of 100, to a level at which there is no apparent deterioration of the image quality due to microscope vibrations. A side-loading specimen stage to fit within the polepiece gap (J. H. Lucas, Rickling, Essex) provides x and y tilts of $\pm 30^\circ$, and some z motion. The rotational symmetry of optical diffractograms taken from micrographs of amorphous materials indicates that specimen drift is not generally limiting performance. High resolution operation can commence immediately after specimen exchange, which takes less than 3 minutes and uses a locally-produced airlock mechanism.

During the commissioning phase of the construction programme, most operation has been at an accelerating voltage of 500 kV. A recent micrograph of a thin film of amorphous carbon recorded at this operating voltage at the optimum defocus has been analysed using a small local computer system¹⁵. This analysis showed continuous information transfer, without oscillations in phase, to a resolution of 0.22 nm, indicating that the microscope is indeed capable of providing image details of atomic dimensions. Micrographs can then be interpreted directly to this resolution without recourse to lengthy calculation and prior knowledge of the specimen structure. Details of

atomic configurations, particularly in the region of crystal defects, can be obtained without complications in image interpretation due to phase reversals. This is to be contrasted with the best commercial 100 kV electron microscopes where the interpretable resolution is limited to greater than 0.28 nm (ref. 16). Lattice fringes as fine as 0.102 nm have also been recorded with this microscope at 500 kV—these are indicative of the instrumental stability and resolving power.

The micrograph reproduced in Fig. 1 is one of a through-focal series of images which shows a small gold particle supported on a thin carbon film. It was recorded at the optimum defocus, so that image features observed, such as the configurations at each of the twinning planes, can be interpreted in terms of projections of atomic columns. However, it should be noted that without detailed calculations, based on an accurate knowledge of particle thickness, it is not possible to ascribe exact positions to the columns¹⁷. The structure image in Fig. 2 shows the complex inorganic oxide $\text{Ti}_2\text{Nb}_{10}\text{O}_{29}$. This material has been previously studied in detail at 100 kV (ref. 18) but not apparently at any higher voltage, although the isostructural $\text{Nb}_{12}\text{O}_{29}$ has been observed at high resolution at an accelerating voltage of 1 MV (ref. 19). The finest specimen features observed at 100 kV in $\text{Ti}_2\text{Nb}_{10}\text{O}_{29}$ which can be interpreted directly in terms of its known structure are the cation positions at the centre of the corner-shared metal-oxygen octahedra which are separated by 0.38 nm. The present image shows much finer interpretable detail, in particular resolving the cations in the edge-sharing octahedra along the regularly-spaced crystallographic shear planes. In a b -axis projection, the centres of these octahedra are separated by only 0.19 nm.

These initial results, and the measured contrast transfer function, indicate that this 600-kV electron microscope has an atomic resolving power, and can be expected to yield substantial and significant information about structures and defects at the

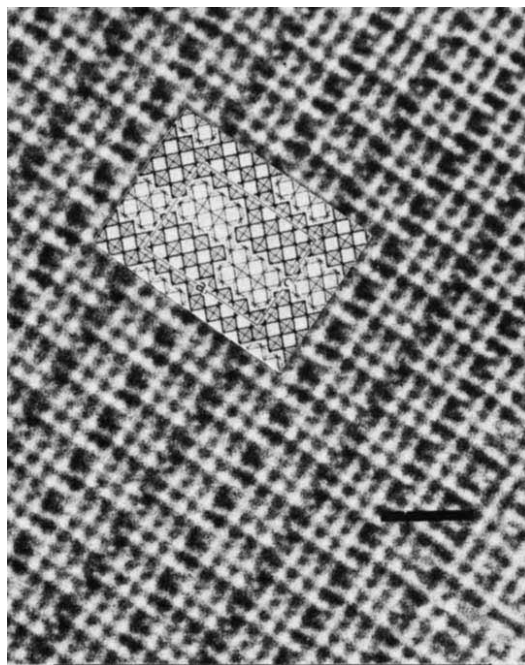


Fig. 2 A structure projection image of the complex inorganic oxide $\text{Ti}_2\text{Nb}_{10}\text{O}_{29}$ in a b -axis projection, recorded at 500 kV at the optimum defocus. As shown in the insert model, this material consists of 3×4 blocks of corner-sharing metal-oxygen octahedra, with the central cations separated by 0.38 nm. These blocks, which are at two levels within the unit cell, are joined by edge-sharing octahedra along the regular crystallographic shear planes, with octahedral centres separated by 0.19 nm in this projection. Near the crystal edge, the micrograph can be interpreted directly in terms of this structure although, in thicker regions, a slight misalignment of the crystal is indicated by lack of mirror symmetry. Scale bar, 2 nm.

atomic level for a wide range of amorphous and crystalline materials, covering many scientific disciplines. Applications have already commenced; there is good agreement between calculated and observed images of silicate minerals²⁰. Other materials under investigation include amorphous metals, multiply-twinned particles, and intercalated graphite catalysts.

The Cambridge University 600-kV high-resolution electron microscope has been constructed with major financial assistance from the SRC. We thank R. H. Pryor and his staff for their assistance, Drs P. W. Hawkes, M. E. Haine, W. D. Riecke and Mr D. A. S. Rose, and many others, who have contributed to the success of the project, and Dr S. Iijima for providing the $\text{Ti}_2\text{Nb}_{10}\text{O}_{29}$ specimen.

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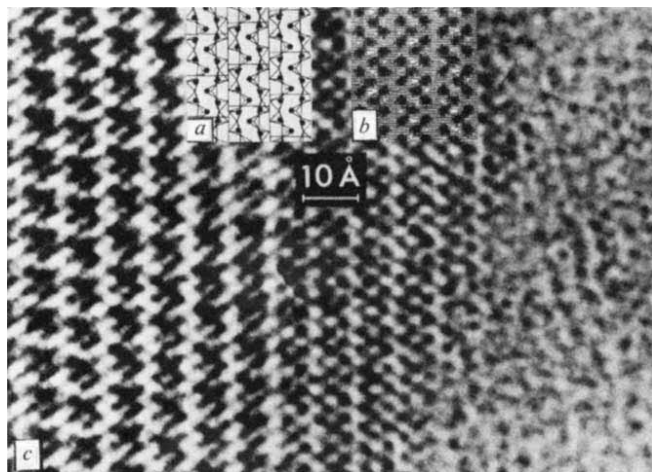


Fig. 1 *a*, Projection down *z*-axis of the idealised structure of wollastonite showing disposition of corner-sharing tetrahedra and Ca^{2+} ions (filled circles). *b*, Computer simulated image (see text and ref. 8). *c*, High resolution micrograph, taken in conditions specified in text.

Currently available 100 kV commercial electron microscopes, fitted with conventional (30°) goniometer stages, cannot structurally resolve (that is, form many-beam images in which each beam has the correct relative phase with respect to the others) interatomic distances of less than ~ 3.2 Å. (It is, of course, possible to obtain lattice fringes separated by less than 1 Å, but these carry only partial structural information.) Consequently most solid-state chemical work carried out using electron microscopy has until now involved structures based on the linkage of corner-sharing octahedral coordination groups, in which the ion exhibiting the sixfold coordination (for example W or Nb) is largely responsible for the scattering contrast seen in the projected image (of a sufficiently thin specimen). The 'block' and 'shear' structures, where the separation distances of the heavy cations are >3.5 Å are good examples^{1–6}.

Silicate minerals, which encompass a wide diversity of topologies that arise from various ways of linking SiO_4 tetrahedra, constitute important tests for the methodology of high resolution electron microscopy, principally because there are good reasons for suspecting that hitherto unknown structures can be revealed by direct, real-space examination of minute regions (encompassing some 10^4 – 10^6 units cells) of naturally occurring materials and their synthetic analogues. Indeed, new topologies (such as multiple-chain structures related to the amphiboles) have recently been identified^{7,8} by comparing computed and observed images of ultrathin specimens in various electron-optical conditions. Furthermore, silicates serve as stringent test specimens for high resolution microscopy because: the central tetrahedrally coordinated atom (Si or Al) displays little inherent contrast in comparison to its surrounding atoms, and the inter-silicon distances are significantly smaller (~ 2.8 Å) than comparable previously imaged distances. Also, the spaces between anionic chains in the silicate network are occupied by cations such as Na^+ and Ca^{2+} , which have scattering powers comparable with those of the atoms in the silicate (or aluminosilicate) macro-anions. Therefore, the interpretation of image contrast is less straightforward and certainly less reliable on an intuitive basis than the contrast displayed by the 'shear' and 'block' structures.

Observations of the pyroxenoid mineral, wollastonite (CaSiO_3) have been made with the Cambridge University 600 kV high resolution transmission microscope^{9,10}. Micrographs demonstrating its atomic resolving power are shown in the preceding paper¹¹.

The initial results closely match previously computed⁸ images for wollastonite. The structure, as deduced from X-ray work¹² on ordered specimens, consists of infinite one-dimensional

Individual silicate chains in wollastonite by high resolution electron microscopy

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Chemists and mineralogists often have to elucidate the crystal structure of new materials even when conventional X-ray methods are not applicable. If, for example, long-range order is not extensive or the amount of material available for examination is minute ($<10^{-7}$ g) the classical techniques of X-ray or neutron diffraction cannot be used. In such circumstances, direct structural determination using high resolution electron microscopy is, in principle, possible. To achieve worthwhile information, however, a point-to-point resolution of 2.8 Å or better is desirable, if the structural details of most oxides, halides, chalcogenides and various oxysalts of metallic and semi-metallic elements are to be established. We demonstrate here how high voltage, high resolution electron microscopy confirms the structure of a representative silicate mineral, showing the potential of the technique for the elucidation of locally abnormal (at the subunit cell level) structures.

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chains of corner-sharing SiO_4 tetrahedra (Fig. 1a). Si-Si distances within the chains are 3.01 and 3.15 Å, but in projection down the z -axis, these reduce to 2.71 and 3.01 Å. Note that in this projection the Ca^{2+} ions which link adjacent chains more or less coincide with the centres of the SiO_4 tetrahedra, and have a separation of 2.9 Å. Computer simulations indicate that a structural resolution of better than 2 Å is required to reveal pairs of chains and the clearly identifiable calcium ions. The computer-simulated image⁸ (Fig. 1b), in the same projection as Fig. 1a, was calculated using the thin phase grating approximation ($\Delta z = 10$ Å) with instrumental parameters: accelerating voltage, 600 kV; coefficient of spherical aberration (C_s) 3.3 mm; defocus of 730 Å; and giving a first zero on the phase contrast transfer function of 1.81 Å. The fine structure of the silicate chains is clearly resolved in this simulation, groups of three SiO_4 tetrahedra, and their attendant Ca^{2+} ions, being readily visible.

Results obtained with an actual specimen of wollastonite in the Cambridge microscope can now be compared with the

computer simulation. From optical diffraction studies^{13,14}, we estimate that information in micrographs such as Fig. 1c extends to 1.44 Å at the edges of the crystal, where the thin phase grating approximation is observed to hold. This micrograph is from a through-focal series of a wedge-shaped crystal of wollastonite, taken with an accelerating voltage of 500 kV with $C_s \sim 3.5$ mm. The phase grating approximation was observed to be valid within a 50–60 Å region at the periphery of the crystal and within this area the observed arrangement of SiO_4 tetrahedra closely resembles that of the computer simulation.

These results show that the prospects of directly investigating the ultrastructure of a wide range of partially ordered minerals and inorganic solids^{2,8,15,16} (borates, phosphates and other classes mentioned earlier) are very encouraging. Such studies of a range of silicates are now in progress.

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A projected potential image of a defect in an ordered alloy?

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The high-resolution performance of 100-kV transmission electron microscopes has improved dramatically in the past few years. Use of an objective lens with a spherical aberration coefficient C_s of 0.7 mm and a low incident beam divergence ($\alpha = 0.2$ mrad) allows point-to-point resolutions shown by Bursill and Wood¹ to be ~ 1.6 Å. These authors, however, distinguish between this 'instrumental' resolution and the resolution which can in principle be used for direct structural resolution and interpretation—only slightly better than 3 Å in the above conditions. The function $\sin \chi$, the part of the lens transfer function most directly dependent on the phase changes χ of the electrons as a function of lens defocus and spherical aberration, shows rapid oscillations for spacings smaller than ~ 2.8 Å even for the optimum defocus condition for which $\sin \chi$ is apparently -1 over a maximum range of spacings¹. Thus even if point-to-point resolutions in the 2–3 Å range are obtained, by careful limitation of the beam divergence, the images can only be related to the structure by very careful comparison with computed images. It is this fact and the limited improvement to be expected on any further reduction of C_s , as well as the severe geometric limitations imposed on specimen manipulation in

such lenses, which has led to the approach of using increased accelerating voltages with a concomitant reduction in electron wavelength, λ : the above 'structure resolution' limit is approximately $0.7 C_s^{1/4} \lambda^{3/4}$. Most of the high-resolution work to date has concentrated on the examination of materials in which there are strong projected charge distribution periodicities at relatively coarse spacings. To obtain further information on the difficulties involved in the examination of defects in metals (with generally smaller spacings of the important charge projections) we have studied the degree to which images of the ordered alloy Ag_3Mg can be related to naive descriptions of the projected charge distribution.

The $\text{Nb}_2\text{O}_5\text{-WO}_3$ system is one in which relatively coarse spacings arise. Iijima² has obtained particularly beautiful micrographs of this system which are clearly directly interpretable. Other work has been done on silicon and germanium and very good apparent resolutions were obtained by Izui *et al.*³ on silicon. While the images are very similar to those naively expected for atomic resolution at 1.36 Å along [100] in the [110] projection the work of Spence *et al.*⁴ and Bursill and Wood¹ suggests that the real instrumental resolution was closer to 1.6 Å, the figure arrived at by the latter authors in their examination of Ti_6O_{11} . This oxide is triclinic and while in the [111] rutile projection discussed by Burstill and Wood¹ the Ti atom column spacing is generally 2.96 Å, near the crystallographic shear planes (CSP) which the material contains at 9.6 Å spacing the projected separation is considerably less. The experimental and computed images are thus of particular interest as the image contrast is complex and shows rapid variation with defocus in a way which is more characteristic of the behaviour to be expected near defects in metals where the symmetry of the local projected potential can be similarly complex.

The minimum spacing of the columns of Mg atoms in the ordered alloy Ag_3Mg (when viewed in the 010 direction (Fig. 1)), is 4.0 Å and thus, given the difference in atomic number

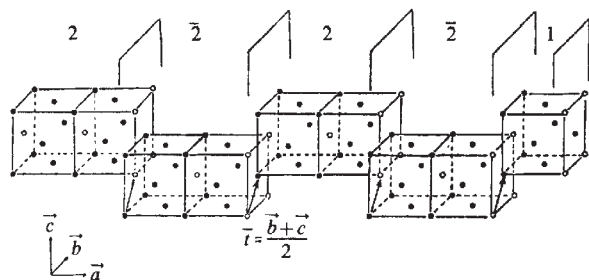


Fig. 1 Diagram, showing the displacements associated with a $(2\bar{2}2\bar{2}1)_2$ long period structure with a repeat in the a direction of 72 Å. ●, Ag; ○, Mg.

between Ag and Mg, images showing the characteristics to be expected for the projected charge distribution can be obtained with relative ease. The material also contains periodic antiphase boundaries (PAPB) which have been shown to be strictly planar with 100 normal⁵, the translation being $(b+c)/2$. The spacings of the important charge projections at these boundaries as viewed in the 010 direction thus provide an interesting test situation for the appearance of high resolution images. As a function of the degree of stoichiometry the alloy also shows a long period structure apparently controlled, at least over a range of composition, by the atomic ratios⁵ with regular insertion of single-cell displacements in a repeat which otherwise shows PAPB every two cells. A diagram of an example of such a structure described, on Fujiwara's⁶ notation, as $(2\bar{2}2\bar{2}1)_2$ with a repeat in the a direction of 72 Å is shown in Fig. 1. Specimens with structures of this general type show occasional imperfections in the long-period arrays in which the apparent position of the single cell part of the array is displaced (for example, $2\bar{2}1\bar{2}2 \rightarrow 2\bar{2}2\bar{1}2$). In an analysis of the way in which the structure itself arises it is of particular interest to determine whether defects of this type are purely displacive or associated as well with progressive changes in the Mg-to-Ag ratios in the relevant columns. Can this question be answered by examination of

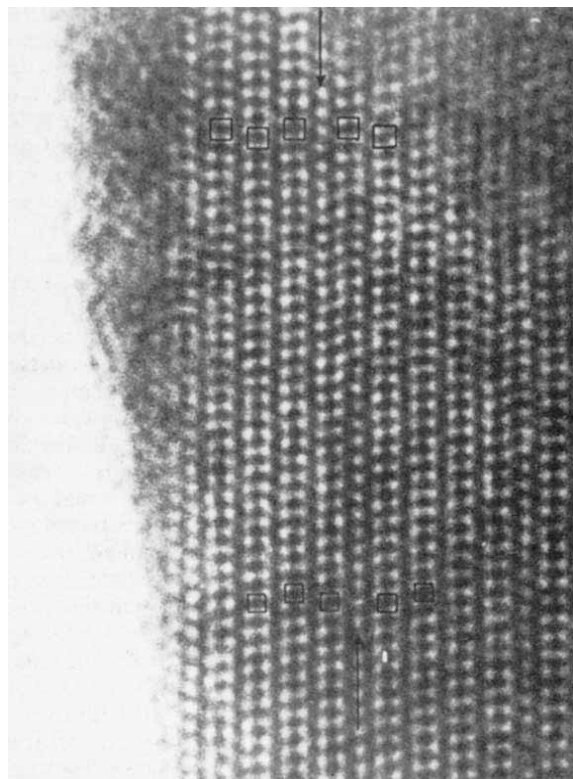


Fig. 2 Axial electron micrograph of a thin region where the position of the single cell width is changed in the long-period structure. The structure is as shown in Fig. 1.

images showing characteristics away from the defect as might be expected, on a naive interpretation, in terms of the projected charge density?

An example of an image of such a defect in the ordered structure is shown in Fig. 2. The micrograph formed part of an extended through focal series. The series was obtained in axial conditions, the specimen being accurately oriented at 010 in a top-entry JEOL tilting holder, in a JEOL 100CX microscope, though with the specimen in a slightly lowered position where C_s is approximately 2.5 mm rather than 3.3 mm as it is for the normally used position in the relatively low resolution pole-pieces of the standard objective lens of this microscope. While a pointed filament was used with a nominal beam current of less than 10 μA and a gun bias sufficient to prevent space charge energy spread limitations in the gun, the images were obtained using overfocussed illumination with $\alpha \approx 0.5$ mrad. This figure is high and for optimum instrument resolution α should normally be limited to a value less than half this figure: as is well known⁷ the envelope function for $\sin \chi$ is controlled not only by the chromatic aberration but also by a combination of the chromatic and spherical aberrations with the convergence and under normal circumstances it is the convergence which is most important.

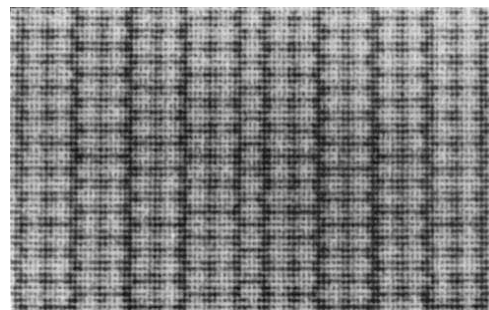


Fig. 3 Image simulation for the model structure shown in Fig. 1 under the experimental conditions used to obtain the micrograph in Fig. 2. C_s , 2.6 mm; Δt , 3,040 Å; specimen thickness, 80 Å. A polynomial expansion technique was used and further details of this and other comparative simulations for a variety of thicknesses will be published elsewhere⁸.

At first sight the image (Fig. 2) shows features, at least away from the defect, which might be interpreted directly in terms of the projected charge density: the regular $(2\bar{2}2\bar{2}1)_2$ form of the ordering may be clearly visualised. Furthermore in the relevant region the through focal series showed only images of this general type, or ones showing minimal contrast. Further back in the foil a variety of features were observed showing rapid variation with defocus. In fact two other images in the series showed the features characteristic of Fig. 2, both with reversed contrast but with minor differences. Image calculations have been completed which match the observed changes in contrast and an example of such a computation appropriate for comparison, with Fig. 2 is presented in Fig. 3. While these calculations will be discussed in more detail elsewhere⁸, the simulations and optical diffraction patterns obtained from the contamination at the edge of the foil showed that the three relevant images corresponded to defocus situations at which the 4 Å spacing was optically transferred in the 3rd, 4th and 5th reversals of $\sin \chi$ respectively. The calculations, for an arbitrary but not unreasonable thickness of 80 Å, also demonstrated, however, that the amplitude and phase changes of the various diffracted beams associated with the periodicity of the ordering should have been sufficiently high to make any application of a concept based on the weak phase approximation totally inappropriate. We thus have the intriguing situation that the gross 4 Å periodicity, characteristic of the projected charge distribution, is readily visualised partially because of the relatively high beam convergence reducing the amplitude of the transfer function for the lower spacings readily seen in thicker foils.

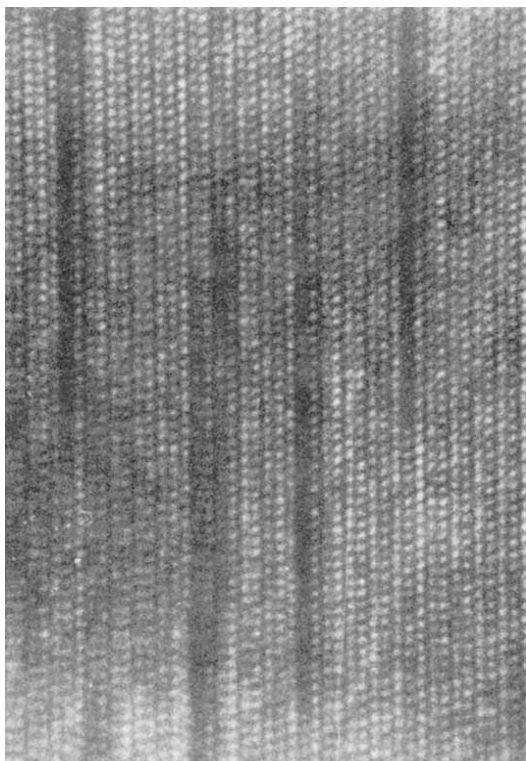


Fig. 4 Axial electron micrograph of a relatively thick region (compare with Fig. 2) at a defocus at which the structure may be visualised. The strong contrast at the defects at this thickness is a clear indication that application of the weak phase approximation would be inappropriate.

Accordingly, although the image in the region of the defect shows none of the features associated with strong diffraction, as are seen in thicker foils (Fig. 4) (see for example ref. 9) and which would normally warn one against any direct interpretation, it is clear that the regular displacements of the apparent projected charge columns near the defect have no simple interpretation. Furthermore the interpretation given here suggests that even with an objective lens with $C_s = 0.7$ mm and low beam convergence no simple interpretation would be possible at the defect not only because the relevant spacings ($2 \text{ \AA}$) would still lie beyond the first zero in the transfer function but also, and as importantly, because the amplitude and phase changes of the diffracted beams would be too high for the weak phase approximation to be relevant. We have thus demonstrated that in perfect crystals the use of a moderate beam convergence coupled with careful tuning of the transfer function allows images to be produced which, given comparison with computation, bear simple relations to the projected potential despite the main features of the image being transferred well beyond the first zero in the transfer function. That this is so does not, however, allow such an interpretation of a defect and the fundamental question of whether or not the defect is essentially displacive or diffusional will probably be answered only with the advent of a direct structural resolution of $2 \text{ \AA}$ as may be possible at higher kilovoltages.

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High resolution imaging of amorphous materials

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The important role of electron microscopy in the study of amorphous materials is in the examination of the local variation in structure, whether inherent or caused by deformation, irradiation or the nucleation of crystallisation. This requires that the image contain information about local spacings and their variability at the atomic level even if only in relation to a projection. Until recently commercial instruments could not do this for spacings less than about 0.3 nm , and material scientists have either concentrated on the interpretation of coarser structural irregularities in amorphous alloys or used indirect methods to get round the lack of resolving power of the objective lens of their microscopes (see ref. 1 for review). The interpretative dangers of the most obvious approach of using tilted illumination conditions are now well understood^{2–5}. While the prospects for dark field interference techniques are a little more promising⁶ the essential problem of a nonlinear transfer function remains⁷. More interesting, though with other limitations, is the use of hollow cone illumination⁸ with axial transfer^{8–11}. Here we present high resolution, axial illumination electron micrographs of such materials with detail at spacings less than 0.2 nm and discuss the difficulties involved in the interpretation of such images.

A JEOL 120CX with a strong objective lens ($C_s = 0.7 \text{ mm}$) was used to obtain images of amorphous materials in the axial mode showing detail down to 0.17 nm and thus potentially capable of interpretation at the atomic level. This might initially suggest that microscopists should be able to tackle the types of problem mentioned above immediately. However in order to understand how images of, for example, deformation bands may be interpreted it is essential to examine first the extent to which detailed information can be gained on atomic coordination in an 'averagely uniform' amorphous material. Thus, after describing our results, we discuss the extent to which this may be done for the technique used.

The technique of tuning the defocus to show up particular spacings in the image is well known for crystalline objects as is the method of choosing a defocus such that the minimum of the phase change covers a broad band of spacings of interest giving them all phase changes approximating to $(2n+1)\pi/2$. Shifting the 'broad band' transfer interval, in this manner, as the spacing examined gets smaller, becomes increasingly important, particularly because it is here that the transfer is least affected by the beam convergence. We have essentially applied the same arguments to the study of amorphous materials and show that, provided the image resolution is not limited by chromatic aberration, the broad band transfer may be tuned to the spacings of interest for a given material down to spacings of $\sim 0.2 \text{ nm}$.

As examples of images of amorphous materials we show micrographs of phosphorus (Fig. 1) and an FeNiB alloy (Fig. 2). The former image was obtained at 120 kV with a defocus of $\sim 170 \text{ nm}$; the latter, at 100 kV , with a defocus of $\sim 250 \text{ nm}$. The convergence angle of the incident illumination, as suggested by the central spot in the electron diffraction pattern was less than 10^{-3} radians, and a pointed filament was used at a beam current of approximately $8 \mu\text{A}$. The images were recorded at

magnifications of approximately 500,000 as calibrated using graphitised carbon test specimens with an error of less than 5%. The optical diffraction pattern of the 120 kV calibration image shown in Fig. 1b may be compared directly with that from the phosphorous image in Fig. 1a.

It is clear that, in the specified conditions, the optical diffraction pattern of the FeNiB alloy demonstrates transfer down to ~ 0.2 nm in the amorphous region. The spots in the diffraction pattern (Fig. 2 inset) are associated with the patch of regularly spaced fringes in this particular image area which are at a spacing of approximately 0.19 nm. It is tempting to suggest that the alloy contained some microcrystalline regions in an amorphous sea, or at least increased order, but it is also possible that we are seeing the first stages of crystallisation under the beam. The number density of such regions in the electron irradiated area is, however, small and this particular patch was present when the first micrograph was taken which was within 30 s of irradiation. Since crystallisation at the 1.0–2.0 nm level is normally only visible in the conditions used after about 10 min, it is possible that it was present before irradiation. The point of interest is, however, that the first ring in the electron diffraction pattern of this amorphous alloy is centred at a radius of about 5 nm^{-1} . Thus, taking the optical diffraction pattern to be a fair indication, the image of the amorphous region ought to contain information about the local atomic arrangements or at least their projection at this level of resolution. Since the electron diffraction pattern of amorphous phosphorus indicates that there is strong scattering associated with spacings around 0.5–0.6 nm and 0.23 nm the image in this case ought to be even more easily interpreted: at the higher kilovoltage the optical diffraction pattern (Fig. 1b) indicates transfer to 0.17 nm.

We must now, however, ask whether or not the optical diffraction pattern is a fair guide to the point to point interpretable information in the micrograph. Consider the appearance of optical diffractograms of axial lattice images. If the crystal is finite, subsidiary maxima appear at $2g$ whether or not these fringes are present in the image. Again, even if these fringes are present they can arise through the interference of phase correlated scattering between $+g$ and $-g$ Bragg reflections and thus may not be indicative of transfer at the angles corresponding to $2g$. Lattice fringes with 0.1 nm spacing at the 001 normal of a gold crystal can be obtained axially but this is indicative of transfer corresponding to spacings of 0.2 nm. In the same material the 0.14 nm 220 lattice fringes may be obtained axially at positions away from the normal where only this systematic row of reflections is strongly excited. This does indicate transfer at this inverse spacing. It is, however, well known that this is much easier than demonstrating equivalent transfer for an amorphous material because relatively larger regions scatter coherently in a crystal than in an amorphous specimen.

The most worrying aspect of the points raised above is that the spacings imaged for the amorphous material, as indicated by the optical diffraction pattern, could result from the equivalent of

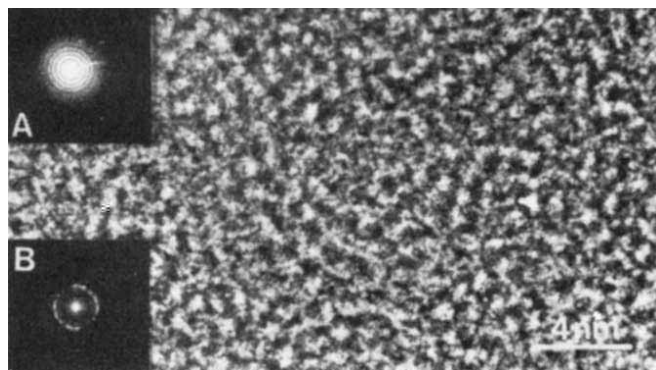


Fig. 1 Image of phosphorus obtained axially at 120 kV. Defocus ~ 170 nm. Insert *a* is an optical diffractogram showing transfer to approximately 0.17 nm. Insert *b* is from graphitised carbon on the same scale and shows transfer at 0.34, 0.21 and 0.17 nm.

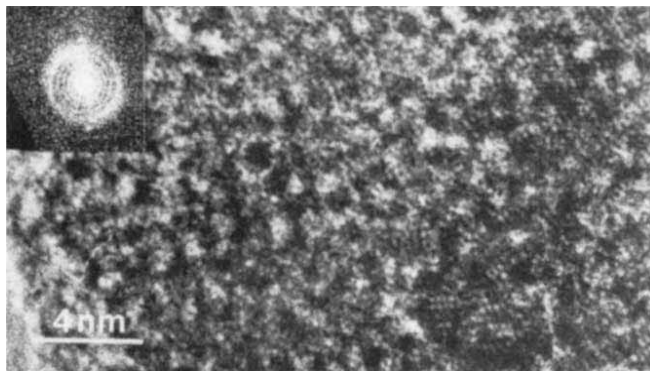


Fig. 2 Image of an FeNiB alloy obtained axially at 100 kV. Defocus ~ 250 nm. The optical diffractogram shows the transfer of the locally crystalline region at 0.19 nm (the scale is different from Fig. 1).

$+g$; $-g$ interferences as described for a crystal. However, the phosphorous used was <10 nm thick and this explanation does not seem likely. That locally etched regions appear to show structure at lower visibility than elsewhere can also be explained on a solely first order scattering approach. We are comparing transfer function behaviour for a variety of materials as a function of their thickness to get more information on this point, as well as on the extent and lack of zeros in the optical diffraction pattern at the smaller spacings as a function of the convergence employed.

With the provisos mentioned above, the results described suggest that suitable analysis of a through focal series of images with the deconvolution of the effect of the transfer function should be possible¹², at least down to spacings at which the ring structure of the transfer function may be seen in the optical diffractograms (~ 0.2 nm). While recording the images requires some care in using a relatively low beam current, the low C_c of the objective lens of the JEOL 120CX means that under normal circumstances the most important experimental aspects are to keep a low beam convergence and to tune the broad band of the transfer function to the spacings of interest.

Note that images of the type described here, where the relevant resolution is attained at relatively large values of defocus, and concomitantly chopped transfer, require considerable computational analysis before a full projection of the amorphous structure may be visualised. (It is extremely difficult to do this without recourse to the weak phase approximation and it is becoming increasingly obvious^{13,14} that this is not very realistic even for the extremely thin specimens normally examined (<10 nm) unless they are of low atomic number.) If the weak phase approximation is not valid then the computation of the relation between the projected structure and the image is much easier if there is continuous transfer out to the lower spacings of interest for the given specimen. This can be done most easily by increasing the accelerating voltage.

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Unsaturated water flow within porous materials observed by NMR imaging

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Wetting, drying and permeation processes in various porous materials are of special interest to soil science and agriculture, building science and chemical engineering. Direct experimental study of unsaturated flow within such materials is hampered by the difficulty of detecting with precision changes in their internal water content distributions. In laboratory work on soils gravimetric sampling is widely used¹. However, this is a destructive method which interferes with water flow processes and in any case is not easy to apply accurately to rigid materials such as permeable rocks, ceramics and building materials. γ -ray attenuation is the only established non-destructive instrumental method in laboratory use^{2,3}. We report the first use of a nuclear magnetic resonance (NMR) imaging technique to monitor the dynamics of the internal water content distribution in several porous inorganic materials during capillary inflow.

The NMR imaging technique allows NMR signals to be isolated selectively from individual volume elements within a structure containing NMR-active nuclei. The major aim of work on this technique is to develop an imaging method for use in medical diagnosis, complementary to existing methods such as X-ray tomography. A description of our method of NMR image formation and examples of its medical use have been given elsewhere⁴⁻⁶. However, the technique is applicable to the study of the distribution of nuclear density within any non-metallic material. In the present case, the hydrogen nuclei (protons) of mobile water molecules within the pores of the material give rise to the NMR signal of amplitude proportional to the water content in defined volume elements.

Our apparatus uses an iron-cored electromagnet generating a field of 0.7 T, corresponding to a proton resonance frequency of 30 MHz. The magnet has a horizontal cylindrical sample compartment of diameter 80 mm and length 600 mm. This

configuration is well suited to the study of the movement of the wetting profile during horizontal capillary absorption of water into long rectangular specimens with sealed sides (approximating to one-dimensional absorption into a semi-infinite solid). Subsequently redistribution and drying processes may be observed.

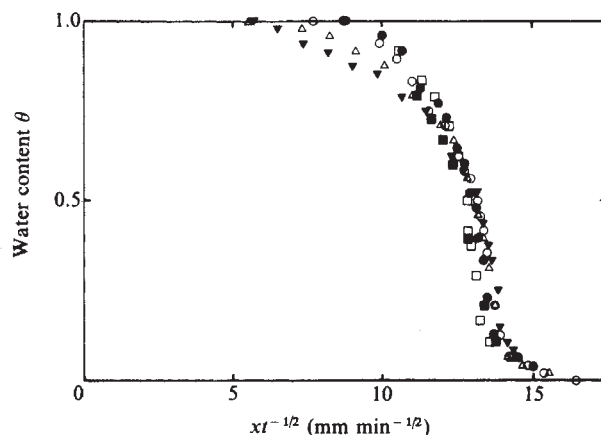


Fig. 2 Water content profiles of Fig. 1 replotted against $xt^{-1/2}$ ($= \phi$) to form a θ - ϕ master curve.

The NMR signal was collected from a magnetically defined thin slice of the specimen transverse to the direction of flow. The complete water content profile was obtained point by point by stepping the specimen in the direction x through the defined slice, enabling the water content in the slice to be determined at a number of distances from the inflow face ($x = 0$). The time needed to measure the NMR signal amplitude from each slice was about 10 s; thus a complete profile could be obtained in about 5 min. Figure 1 shows a family of water content profiles determined during capillary absorption into a gypsum-lime plaster bar. The signal amplitude is scaled to correspond to the range 0–1 in θ , the reduced water content. ($\theta = (u - u_0)/(u_1 - u_0)$ where u is the volumetric or gravimetric water content and subscripts 1 and 0 indicate nominal saturated and dry states⁷. All materials used were initially air-dry; thus $u_0 \approx 0$ and $\theta = u/u_1$.) The shape of the leading portion of the profile (at the lowest water contents) is sensitive to the defined slice thickness. Subsequent measurements showed a steeper intersection with the x axis (see Fig. 3), and a resolution of < 2 mm.

Much of the analysis of unsaturated flow developed in soil physics⁸ and more recently applied to water transport in building materials^{7,9} is based on the extended Darcy equation¹⁰. If valid, this means that one-dimensional horizontal flow is described by

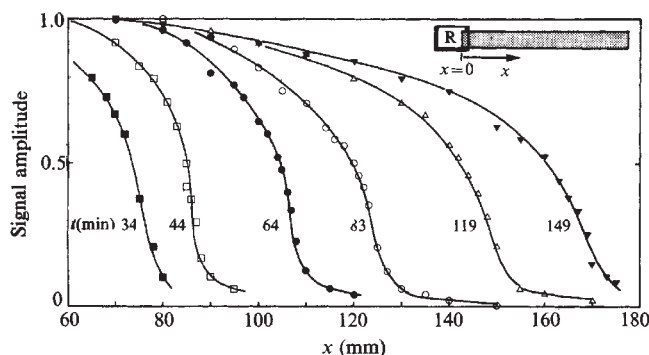


Fig. 1 Water content profiles obtained by NMR during capillary absorption of water by a plaster bar. Elapsed times t are shown. The bar ($235 \times 33 \times 33$ mm) was cast from a mix (1:2 by volume) of a commercial hydrated lime and a commercial retarded hemihydrate plaster (class B, type b2 gypsum building plaster). After setting and drying, all side faces were sealed with an epoxy coating. Inset sketch shows bar with attached reservoir R to supply water to the inflow face during the experiment. Signal amplitude is scaled to the range 0–1, corresponding to a reduced water content (θ) range of 0–1. $u_1 \sim 0.45$ cm³ water per cm³ dry solid.

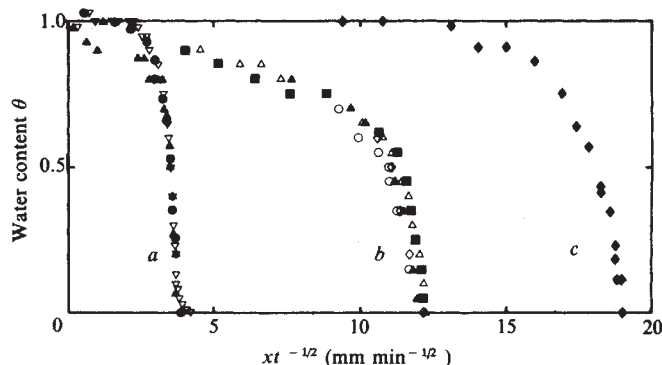


Fig. 3 θ - ϕ Master curves for capillary absorption. a, Portland limestone ($u_1 \sim 0.11$ cm³ per cm³). b, Ordinary Portland cement:lime:sand mortar, 1:3:12 by volume ($u_1 \sim 0.27$ cm³ per cm³). c, Class B, type b2 gypsum building plaster ($u_1 \sim 0.37$ cm³ per cm³).

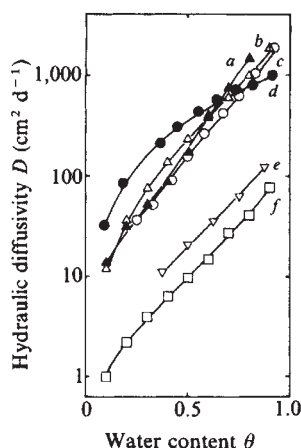


Fig. 4 Hydraulic diffusivity $D(\theta)$ of six materials calculated from NMR capillary absorption profiles. *a*, Cement:lime:sand mortar (1:3:12). *b*, Gypsum building plaster. *c*, Gypsum building plaster + hydrated lime. *d*, Locharbriggs sandstone ($u_1 \sim 0.25$ cm³ per cm³). *e*, Corsehill sandstone ($u_1 \sim 0.24$ cm³ per cm³). *f*, Portland limestone.

the nonlinear diffusion equation

$$\frac{\partial \theta}{\partial t} = \frac{\partial}{\partial x} \left\{ D(\theta) \frac{\partial \theta}{\partial x} \right\}$$

where θ is the water content and D is the unsaturated hydraulic diffusivity, a property of the porous material. This equation has the solution $x(\theta, t) = \phi(\theta)t^{1/2}$ for absorption at a wet face ($\theta = 1$) into an initially dry ($\theta = 0$) semi-infinite homogeneous material. Thus the water-content distance profile advances as $t^{1/2}$, maintaining constant shape $\phi(\theta)$. Figure 2 shows that the profiles of Fig. 1 when replotted against $xt^{-1/2}$ fall approximately on a single θ - ϕ master curve. Figure 3 shows similar master curves obtained from capillary inflow profiles on three other materials.

These results, taken together with the reworking (unpublished) of two sets of γ -ray data^{11,12} on autoclaved aerated concretes and with a number of studies on soils¹³ provide strong support for the applicability of the extended Darcy equation to such porous materials. From θ - ϕ data the unsaturated diffusivity $D(\theta)$ may be calculated for each material by the method of Bruce and Klute¹⁴. Results for six materials are shown in Fig. 4.

Several other wetted porous materials have also been examined. Apparently satisfactory signals were obtained from a clay loam, and from two sand samples. The NMR response from several specimens of fired-clay building bricks and from an autoclaved aerated concrete was extremely weak, indicating an exceptionally short nuclear relaxation time. A calcitic sandstone also gave a very weak response. The factors controlling signal amplitude from different materials have not been investigated.

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Single colloidal crystals

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Colloidal suspensions of highly charged, monodisperse polymer spheres exhibit long-range (crystalline) translational ordering in appropriate conditions of charge, number density, counterion concentration and temperature¹⁻⁵. These 'colloidal crystals', which can conveniently be made to have lattice parameters comparable to or greater than optical wavelengths, offer unique opportunities for the study of the collective static and dynamic behaviour of strongly interacting spherical particles. For example, such systems may readily be probed by relatively simple but powerful light scattering spectroscopic techniques^{6,7}. Furthermore, the ordering itself offers intrinsic advantages, both experimental (for example, fluctuations normally observable only about the k-space origin appear about Bragg spots, with reduced stray light and multiple scattering effects), and theoretical (the simplicity of calculating on the basis of a known rather than only statistically defined structure is well appreciated from experience with atomic solids and liquids). To exploit these ordered structures fully, reliable methods of producing orientated single crystals suitable for light scattering and other optical studies must be developed. For light scattering from bulk samples the primary requirement is that the ratio of interparticle spacing to diameter, a/d , be sufficiently large. This reduces interference by multiple scattering and renders the particle scattering factor nearly constant through those scattering angles $[|k| < (2 \rightarrow 5)2\pi/a]$ where the most useful information concerning crystal structure and dynamics appears. We now describe a technique whereby single body-centred cubic (b.c.c.) colloidal crystals, well suited for light scattering and having particular orientations, may be produced.

Our method is shown schematically in Fig. 1, and may be described as follows. The colloidal suspension (0.109 or 0.234 μ m diameter Dow polystyrene spheres at ~ 0.1 wt% in thoroughly deionised water) is introduced into a rectangular quartz cell of path length 1 or 2 mm (Fig. 1B). A shear flow is created by mechanically rocking the cell about the axis normal to the flat quartz plates which form the faces of the cell. The intensity distribution of light scattered by the spheres in suspension from a weakly focused argon ion laser is observed and photographed on a translucent screen. At zero flow rate the suspension, if suitably deionised, will crystallise. The application of shear flow disrupts the crystallisation such that suspensions which are crystalline at low or zero shear rates adopt a liquid-like, amorphous structure at sufficiently high shear rates (≥ 20 Hz). Figure 2A shows the scattered light intensity distribution from a suspension under shear and it exhibits the diffuse Debye-Scherrer ring typically observed in the static structure factor of liquids of strongly interacting spherical particles. If the shear flow is stopped abruptly (within 1 s), crystallisation begins immediately, with the nucleation of crystallites at a large number of (as yet unidentified) sites in the suspension which grow to fill the entire suspension volume in a few minutes. The result is a polycrystalline array yielding a scattered light intensity distribution typical of crystalline powders (Fig. 2B). Such arrays seem to be stable over periods of several months.

If, on the other hand, the shear rate is only very gradually decreased to zero (over a period of $\sim 10^3$ s), the resulting structure is much more highly ordered, as indicated by its diffraction

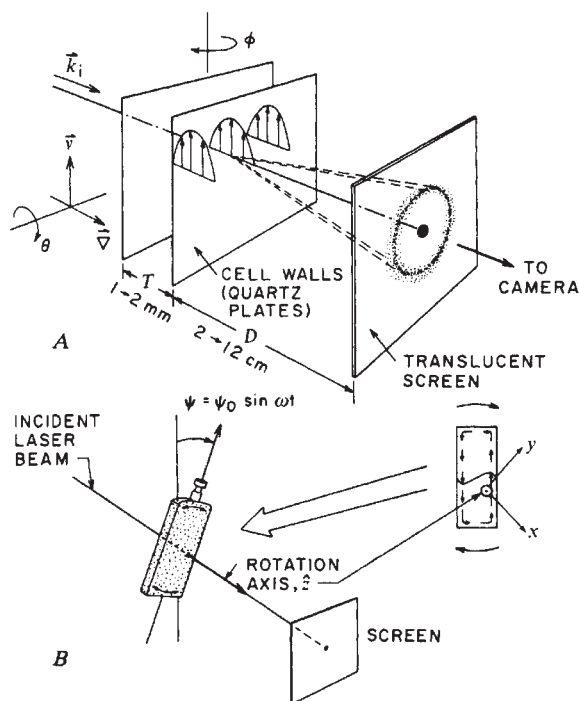


Fig. 1 A, Geometry of the apparatus for the generation and observation of b.c.c. single crystals. B, Details of the sample cell. Samples were placed in quartz cuvettes of dimension $1 \text{ or } 2 \text{ mm} \times 5 \text{ mm} \times 10 \text{ mm}$, with a small amount of mixed bed H-OH ion-exchange resin in the bottom. Variable shear flow was induced by rocking the cell about the z axis ($\psi \equiv \psi_0 \sin \omega t$) at a variable frequency ω . Inertia of the suspension produced the indicated flow pattern relative to the cell, with $\vec{v} \perp \hat{z}$ in the probed region and with the largest velocity gradient having $\vec{v} \parallel \hat{z}$. The $\hat{x}$ and $\hat{y}$ axes in the inset are shown in one of three possible orientations, the $\hat{x}$ axis of crystals making angles of 0° , 60° and 120° to $\vec{v}$.

pattern (Figs 2C, 3A). The study of this pattern and its dependence on the cell orientation angles (θ, ϕ , Fig. 1A) indicates that this structure is an array of two-dimensional hexagonal closest packed (h.c.p.) layers orientated normal to the velocity gradient (z axis, that is, parallel to the quartz plates) and with a set of two-dimensional h.c.p. rows of index 10 parallel to the velocity vector. We believe the stacking of these h.c.p. layers to be nearly random as, on the one hand none of the Bragg spots in the two-dimensional h.c.p. pattern is eliminated by this stacking, but on the other hand the two-dimensional h.c.p. 30 spots are generally of comparable intensity to the others, indicating the lack of registration of the two-dimensional h.c.p. planes in discrete three-dimensional h.c.p. sites. The formation of this and other structures of intermediate order from the liquid as the shear rate is lowered will be discussed elsewhere.

This disordered array of two-dimensional h.c.p. layers is a metastable structure. Within several hours of its formation a new structure appears at sparsely scattered nucleation sites and slowly grows over a period of days to weeks to fill the suspension volume. Figure 3B shows a typical diffraction pattern obtained from this second structure with the cell normal to the incident laser beam ($\theta, \phi = 0$). We identify the second structure as a single crystal orientated as indicated in Fig. 4A, with a set of planes containing a 110 and a 100 axis parallel to the two-dimensional h.c.p. planes of the metastable structure and therefore parallel to the bounding plates. The b.c.c. structure has been observed previously in low-density colloidal crystals⁵. The nucleation process is such that the b.c.c. 110 axis is parallel to one of the three two-dimensional h.c.p. 10 axes, allowing three distinct orientations of the b.c.c. crystals about the z axis. The b.c.c. crystals, once nucleated, grow most rapidly along the z axis to fill the space between the plates and then gradually expand in the plane of the cell, with large crystals growing at the expense of

smaller crystallites. The result, after several days to weeks, is a mosaic of single b.c.c. crystals, each completely filling the millimetre-size gap between the cell walls, and having one of the three preferred orientations about z . In a sample left undisturbed for 2 months after preparation of the h.c.p. structure single b.c.c. crystals as large as $7 \text{ mm} \times 5 \text{ mm} \times 1 \text{ mm}$ were found.

Figure 4A shows the b.c.c. crystal orientation which produces the intensity distribution of Fig. 3B along with the corresponding f.c.c. reciprocal lattice and Ewald sphere for our light scattering geometry with the sample cell normal to the laser beam ($\theta, \phi = 0$). By suitably rotating the sample about the x and y axes some 16 reciprocal lattice points can be brought onto the Ewald sphere to produce Bragg scattering. Figure 3C shows typical results. Here the cell has been rotated about two axes ($\theta = -8.1^\circ$, $\phi = -11.5^\circ$) to bring the 011, 211 and 200 points onto the Ewald sphere ($2\pi/k_i = (\lambda/n)i = 458 \text{ nm}/1.33 = 344 \text{ nm}$). Note the complete absence of 111 and 100 spots, which disappear in b.c.c. diffraction patterns, whereas the observable Bragg reflections index to within a few per cent of those expected for a b.c.c. lattice. Typical cubic lattice parameters were $a = 2.2 \mu\text{m}$, about 10 particle diameters.

The geometry of Fig. 4B indicates that there is no Bragg scattering with the cell normal to the incident beam, so that the intensity distribution of Fig. 3B must arise from displacements of spheres from their positions in a perfect b.c.c. lattice. Visual inspection of this light shows a pattern of fluctuating coherence areas indicating that it is dynamic in character. Preliminary intensity fluctuation spectroscopy studies show a characteristic fluctuation time which increases as the intensity increases, such as when a Bragg reflection is approached. These observations, along with the similarity of the observed intensity distribution to that for X-ray thermal diffuse scattering in the same planes of atomic b.c.c. crystals⁶, lead us to identify this non-Bragg scattering tentatively as thermal diffuse scattering, that is, arising from thermal vibrations of the b.c.c. lattice.

The quality of the b.c.c. single crystals was evaluated by (1) monitoring the presence or absence of spurious Bragg spots; (2) monitoring the spatial distribution of the Bragg reflected light; (3) by the presence of Kossel lines. The b.c.c. crystals, once formed, were extremely delicate. Motion of the cell would invariably lead to distortion and shrinkage of the crystals. Damage produced by motion of the cell, if not too extreme, would anneal out over a period of a few days. In carefully handled samples diffraction-limited Bragg spots from crystals of size $\sim 1 \text{ mm}^3$ could be readily obtained.

In well ordered samples the diffraction pattern exhibited Kossel lines (Fig. 3D). These are light (dark) bands in the diffusely scattered light where rays have been Bragg reflected into (away from) directions corresponding to the Bragg angle for a set of crystal planes. The diffusely scattered light is incident at

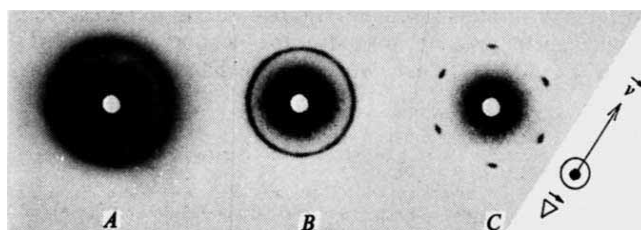


Fig. 2 Photographs of scattered intensity distributions (real images as observed from behind screen). A, Debye-Scherrer ring obtained for a suspension of $0.109 \mu\text{m}$ -diameter spheres translationally disordered by shear at a root mean square shear rate of 20 Hz : ($\theta, \phi = 0$). This suspension is crystalline at zero shear. $T = 1 \text{ mm}$, $D = 2 \text{ cm}$. B, Crystal powder pattern obtained from the suspension of A if the shear is abruptly stopped; ($\theta, \phi = 0$). Intensity distribution obtained from suspension of A if the shear rate is gradually lowered to zero. The shear velocity and gradient directions are indicated. The structure is a disordered array of two-dimensional h.c.p. layers with the two-dimensional h.c.p. planes normal to $\vec{v}$ and having a 10 direction normal to $\vec{v}$ ($\theta, \phi = 0$). $T = 1 \text{ mm}$, $D = 12 \text{ cm}$.

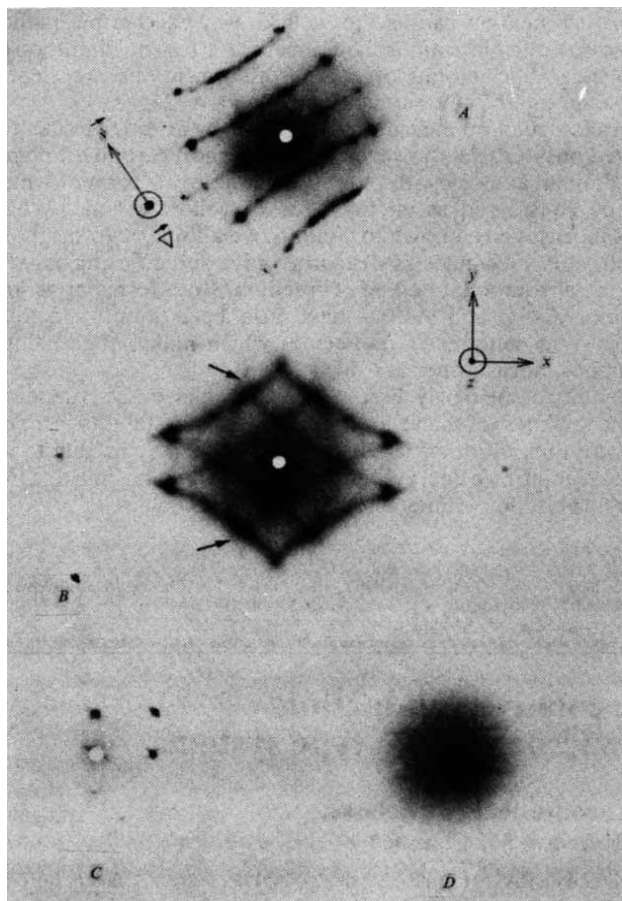


Fig. 3 Photographs of scattered intensity distributions (real images as observed from behind screen). **A** Same as Fig. 2 **C** for $0.234 \mu\text{m}$ diameter spheres [$(2\pi/k_i) = 386 \text{ nm}$]. **B**, Intensity distribution observed for $(\theta, \varphi = 0)$ for one of the three single b.c.c. crystals that can nucleate from the structure of Fig. 3 **A**. This scattered light is thermal diffuse. The Kossell lines (indicated by arrows) are modifications of the thermal diffuse intensity along pairs of parabolas symmetrically arranged as mirror reflections about the origin, each pair arising from a reciprocal lattice vector and its negative [$(2\pi/k_i) = 386 \text{ nm}$]. **C**, Crystal of Fig. 3 **B** rotated to $(\theta = -8.1^\circ, \varphi = -11.5^\circ)$ to yield the 011, 211 and 200 Bragg reflections. The b.c.c. 011 spacing is the same as the two-dimensional h.c.p. 10 spacing [$(2\pi/k_i) = 344 \text{ nm}$]. **D**, Kossel lines obtained by illumination of a single b.c.c. colloidal crystal orientated as in **B** with a diffuse point source made by focusing the incident laser beam onto a thin Teflon sheet as it enters the cell ($d = 0.234 \mu\text{m}$, $2\pi/k_i = 386 \text{ nm}$).

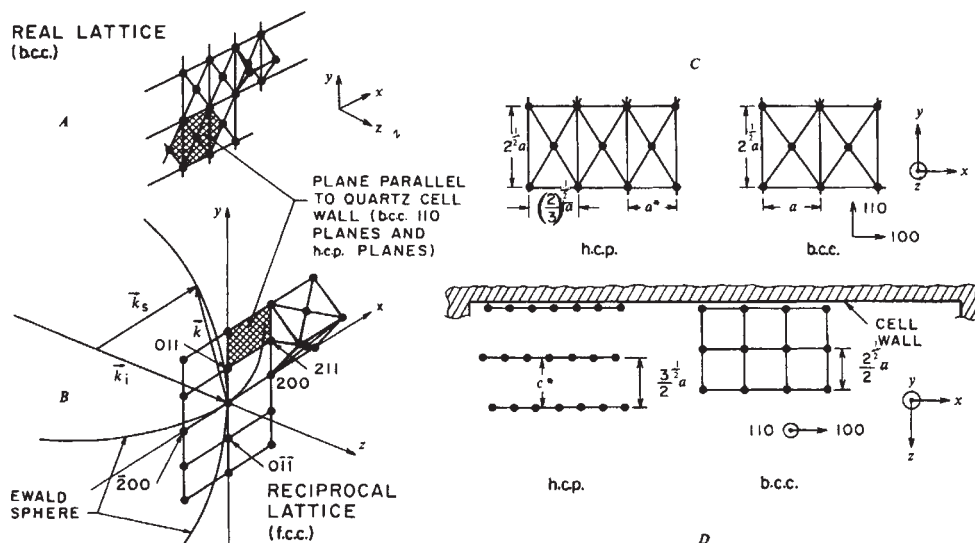
all angles to the crystal so the affected rays lie on a cone coaxial with each reciprocal lattice vector (normal to the crystal planes) and at an angle complementary to the Bragg angle⁹. The Kossel lines occur at the parabolic and hyperbolic intersections of these cones with the viewing screen. They appear in X-ray scattering studies of good single crystals and are a sensitive although qualitative indicator of crystal perfection. Motion-induced distortion of a b.c.c. single crystal which has no apparent effect on the Bragg or thermal diffuse scattering can completely eliminate the Kossel lines, which will gradually reappear over several days if the sample is left untouched. The Kossel lines can be viewed alone by illuminating the crystal with diffuse light from a point source, obtained by placing a thin Teflon scatterer in the focused incident laser beam where it enters the cell. The result is shown in Fig. 3 **D**.

The b.c.c. crystalline structure bears common features with the randomly stacked two-dimensional h.c.p. from which it nucleates, as indicated in Fig. 4 **D**. Both structures are composed of a stack of layers which are parallel to the bounding plates. In the first structure these layers are two-dimensional h.c.p.s. In the b.c.c. structure they are distorted two-dimensional h.c.p.s, having been stretched in only one dimension, that which becomes the b.c.c. 100 axis (Fig. 4 **C**). There seems to be little or no distortion along the other direction (b.c.c. 110 spacing = two-dimensional h.c.p. 10 spacing). The appearance of the b.c.c. is thus accompanied by the disappearance of rows of particles in the two-dimensional h.c.p. layers, perhaps by dislocation motion. If we assume that the overall particle number density remains constant during the transition to b.c.c., the decrease in the number of particles per unit plane area must be accompanied by an increase in the number of planes between the cell walls. For zero density change the two-dimensional h.c.p. layer spacing, c^* , would have to be $(3^{1/2}/2)a$ for a c^*/a^* ratio of 1.83. This somewhat large value may be a result of the shearing which tends to force the spheres into planes normal to the flow gradient. Once the flow is stopped the spheres become too densely packed into layers which are too far apart, and move to form a more energetically favourable structure.

In the conditions of our experiments the crystals were only a few degrees kelvin below their melting temperature, as large room temperature variations would cause partial melting. Recently it has been shown on the basis of an extended Landau theory that, near a weakly first order melting, crystals of spherical particles should exhibit a b.c.c. structure¹⁰. Our observation of a transition from a metastable two-dimensional h.c.p. layer array structure to a b.c.c. structure provides additional evidence to support this argument.

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Fig. 4 **A**, b.c.c. crystal orientation which produces patterns of Fig. 3 **B,C**. **B**, Corresponding f.c.c. reciprocal lattice and Ewald sphere. **C**, Stretching transformation which must occur in the two-dimensional h.c.p. layer during the growth of the single crystal b.c.c. structure. **D**, Randomly indexed h.c.p. layers of spacing $c^* = (3^{1/2}/2)a$ transform into layers at the b.c.c.-indexed positions with spacing $(2^{1/2}/2)a$.



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A new phase in solid state arsenic

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It has generally been considered that in the solid state, arsenic exists as four allotropic modifications—yellow (As(I)), amorphous (As(II)), orthorhombic (As(III)), and rhombohedral (As(IV)). Free energy content is lowest for the rhombohedral form. The other forms are metastable, the yellow form being the least stable, existing only at low temperatures and in the dark¹. It is the yellow form which has been the least studied. Since the effect of temperature, as well as that of irradiation with light or X rays, is to induce amorphisation in yellow arsenic, no X-ray structural data are yet available, and only indirect observations have been available. From these a cubic form has been tentatively assigned to yellow arsenic crystals. Spectroscopic data suggest molecular bonding in the crystal, with the As₄ molecule as the structural unit—a molecule previously observed in the vapour state².

There is little information available on the thermodynamic properties of yellow arsenic. This is relevant to attempts to raise the thermal stability margin, thus opening the possibilities of practical application of the material for silverless photography. We have now designed and built a Calvet-type microcalorimeter for studying phase transitions in thin evaporated films. Using this device for differential thermal analysis (DTA) we have obtained DTA curves showing the existence of a previously unknown reversible (endothermic) phase transition within the metastable As(I) yellow form of arsenic which thus contains two states As¹(I) and As²(I).

The transition curve for As(I) starts at 227 K, reaching a maximum at 230 K. Reversibility was tested by repeated heating-cooling cycles of the calorimeter, the heating being interrupted immediately after termination of endothermic phase transition.

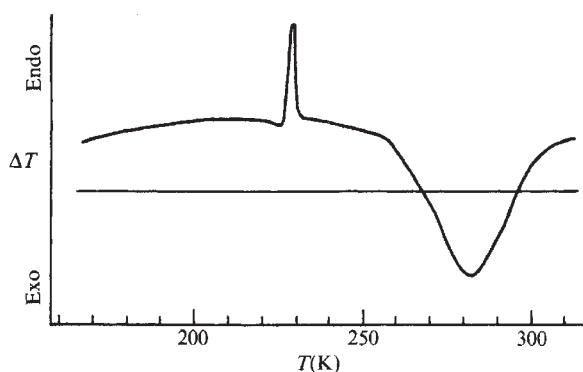


Fig. 1 DTA curve of thin evaporated film of yellow arsenic condensed at 205 K.

Further heating causes the well known exothermic monotropic transition into amorphous arsenic As(II) with liberation of a considerable amount of heat. Both transitions are clearly visible on the thermographic curve shown on Fig. 1.

Direct structural measurements of yellow arsenic have so far proved unsuccessful, however, we can suggest certain estimates based on an analogy with phosphorus, the white form of which can be considered as an analogue of yellow arsenic. White phosphorus is also known to contain two allotropic forms, both based on P₄ molecules as structural units, and differing only by additional rotational degree of freedom. Both forms of yellow arsenic—As¹(I) and As²(I)—have also been found spectroscopically to contain As₄ molecules which makes the analogy with phosphorus rather feasible.

Stability of the newly discovered yellow phase As²(I) depends on the thickness of the yellow arsenic layer, the stability increasing with diminution of layer thickness. This may be due to an increase in surface energy with thinning of layer, leading to a rise in amorphisation temperature.

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Percolative conduction in microemulsion type systems

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In the presence of a suitable combination of surfactants (an alkaline metal soap and a medium chain length alcohol, for example), water and oil type organic liquids can form transparent compounds of low viscosity that have been labelled "microemulsions", with little or no mechanical agitation (spontaneous emulsification) (refs 1–3). Depending on the chemical nature of the surface active agents and the relative constituent proportions, water-in-oil (w/o) or oil-in-water (o/w) systems can be obtained. (Because of the similarity between tertiary solutions of inverted micelles and w/o microemulsions, the term 'inverted micellar solutions' has also been suggested^{4–7}.) Microemulsions have been investigated by many scientists interested in liquid state and surface physicochemistry and by many technologists foreseeing numerous applications in industry^{8,9}. It has been suggested^{9–13} that conductivity and permittivity studies could provide, along with other techniques, valuable information about the structure and phase behaviour of microemulsions which are considered to consist of dispersions between a few tens and a few hundreds of angstroms in diameter globules made up of an inner spherical core surrounded by a concentric shell of mixed surfactant and cosurfactant^{1–10}. The experiments reported here show that the conductive behaviour of certain microemulsion systems can be accounted for using the percolation and effective-medium theories^{14–16} that have depicted transport properties and continuous metal–non-metal transitions in disordered materials with microscopic inhomogeneities associated with, for example, density, composition¹⁵ or bonding configuration¹⁷ fluctuations. This result, which could help in understanding the structural behaviour of microemulsions, is considered in connection with the postulated existence in liquid systems of equilibrium bicontinuous structures, a state described by Scriven¹⁸ as 'related to ordinary liquids as porous media are to homogeneous solids'.

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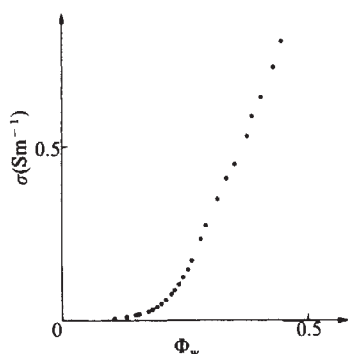


Fig. 1 Variations of σ with Φ_w . The mass fraction of combined surface active agents in the systems was kept at 40%.

Using a Wayne-Kerr B331 autobalance precision bridge, accurate conductivity determinations were obtained at 1.59 kHz and 295 K on w/o type microemulsions involving water and toluene and in which the mass ratio of surfactant (potassium oleate) to cosurfactant (butanol) was kept at 0.5. Unlike previous investigations^{9-12,19}, measurements were carried out run by run, each run corresponding to a definite class of systems characterised with a fixed value of the mass fraction of combined surface-active agents.

Figure 1 shows the variations of the conductivity σ versus Φ_w , the disperse water volume fraction, recorded on microemulsion systems in which the mass fraction p_s of combined surface-active agents was kept at 40%. Similar plots were obtained on systems characterised by different values of p_s , ranging from 30 to 55%. Figure 1 shows that the σ versus Φ_w plot exhibits features characteristic of percolative conduction, the σ experimental values sharply increasing as Φ_w passes a critical value. Kirkpatrick¹⁴ developed, through Monte-Carlo numerical simulations applied to resistor network models, a tractable approach towards percolative conduction phenomena in inhomogeneous media. For conductor-insulator composite materials in which a rigorous percolative conduction is assumed, the effective conductivity σ , zero as long as the conductor volume fraction Φ is smaller than a critical value Φ^P , called the percolation threshold, suddenly takes non-zero values when Φ becomes slightly greater than Φ^P and then increases with Φ . In the vicinity of Φ^P , the dependency of σ on Φ ($\Phi > \Phi^P$) can be demonstrated in the case of three-dimensional systems, by the following scaling law:

$$\sigma(\Phi) \propto (\Phi - \Phi^P)^{8/5} \quad (1)$$

For higher values of Φ , the variations of σ on increasing Φ can be fitted using a relation

$$\sigma(\Phi) \propto (\Phi - \Phi_c) \quad (2)$$

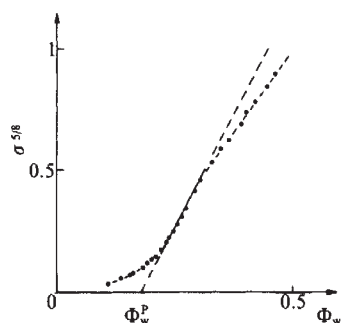


Fig. 2 Determination of Φ_w^P , the water volume fraction corresponding to the percolation threshold, using a scaling law. Specifications as in Fig. 1.

whose range of validity overlaps that of equation (1). It has been shown^{14,16} that the B. B. L.²⁰⁻²² effective medium formula

$$\frac{\sigma - \sigma_2}{3\sigma} = \frac{\sigma_1 - \sigma_2}{\sigma_1 + 2\sigma} \Phi \quad (3)$$

an expression of the conductivity σ of a binary composite as a function of the conductivities σ_1 and σ_2 of the two of its constituents (Φ being the volume fraction of constituent 1) yields for vanishing values of σ_2 (for conductor-insulator mixtures) an equation of type (2)

$$\sigma/\sigma_1 = \frac{3}{2}(\Phi - \frac{1}{3}) \quad (4)$$

in which Φ_c appears to be $1/3$. As mentioned by Kirkpatrick¹⁴, different theoretical values have been suggested for percolation thresholds in three-dimensional systems depending on their geometry. The value $\Phi^P = 0.29$ is of particular relevance to microemulsion studies as it was derived from numerical estimations on a continuum percolation model in which the allowed volumes surrounding percolation sites are assumed to be identical spheres that can overlap and have centres distributed at

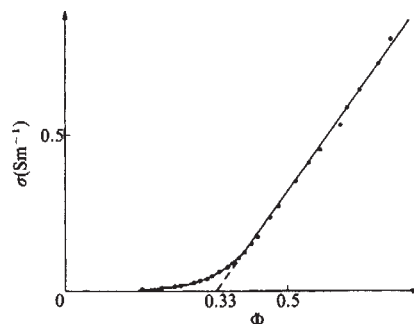


Fig. 3 Variations of σ with Φ , the volume fraction of disperse phase (water cores plus shells of combined surface active agents). Specifications as in Fig. 1.

random. This model is consistent with that inferred from other investigations into microemulsion structure and internal interactions^{1-10,19,23}. In the present study, the percolation threshold was determined in terms of water volume fraction by plotting, in accordance with equation (1), $\sigma^{5/8}$ versus Φ_w . Figure 2 shows that the middle part of the plot can be fitted by part of a straight line whose intersection with the Φ_w axis provides Φ_w^P , the critical water volume fraction whose mean value was found to equal 0.176 ± 0.005 . Comparing this experimental determination with the theoretical threshold value of 0.29 mentioned above yields the ratio of the disperse globule external radius, a , to the water core radius, a_c , namely $(a/a_c) = 1.18 \pm 0.01$. This value is in excellent agreement with that reported by Lagües *et al.*¹⁹ who found, through neutron scattering measurements, $a/a_c = 1.17$ in the case of water-in-cyclohexane microemulsions using SDS and 1-pentanol as the surface-active agents. Lagües *et al.* suggested for their systems a water critical volume fraction of 0.078. Assuming that the value of the ratio (Φ/Φ_w) as determined from that of (a/a_c) remains unchanged at higher water contents, it was possible to plot the conductivity data versus Φ , the volume fraction of the disperse phase (water cores plus combined surfactant shells), to check whether the microemulsion conductive behaviour beyond the percolation threshold could be fitted by equation (4). Figure 3 shows that, for values of $\Phi > 0.4$, the versus Φ diagram is part of a straight line whose intersection with the Φ axis gives $\Phi_c = 0.330 \pm 0.005$, which accords with the value of $1/3$ predicted by equation (4). A similar agreement was observed for the other values of p_s . Moreover, whatever the value of p_s , the values $\sigma(1)$ obtained by extrapolating the σ versus Φ plots at $\Phi = 1$ allow values of the slope factor S to be computed which are in excellent agreement with the theoretical value of $3/2$ in equation (4); for instance, the value 1.31 S m^{-1}

deduced from Fig. 3 yields $S = 1.51 \pm 0.01$. This general result proves that σ_1 can be considered as representative of the conductivity σ_i of the conducting constituent in the systems, before their inversion into o/w type ones. Note that from Figs 2 and 3, below Φ^P , the conductivity is not strictly equal to zero as it should be were a rigorous percolative conduction exhibited by the microemulsions studied. The non-zero residual conductivity below the percolation threshold could be accounted for on the basis of electrophoretic movements of the disperse globules, as suggested by Peyrelasse *et al.*²⁴.

These results show that the percolation and effective-medium theories, that are usually applied to semiconductor-metal transitions observed in inhomogeneous systems and to transport properties in amorphous solids, can be extended to the case of microemulsion electrical behaviour. General descriptions of the topological aspects of percolation phenomena in inhomogeneous media have been given by Kirkpatrick¹⁴ and Webman *et al.*¹⁶. In a conductor-insulator composite, the conducting constituent is divided into isolated clusters as long as its volume fraction Φ remains $< \Phi^P$, bulk conduction phenomena being so inhibited. When Φ reaches and slightly exceeds Φ^P , a certain number of clusters interconnect with each other leading to the formation of a large cluster acting as a conducting path stretched throughout the sample that therefore acquires a non-zero conductivity. When Φ is $> \Phi^P$, the large cluster extends rapidly, by absorbing small isolated clusters which then become less and less, and the sample conductivity increases proportionally to Φ . This scheme may be tentatively applied to the case of microemulsion electrical conductivity, on the basis of the present experimental results. The increase in microemulsion conductivity observed when the water content is raised above the percolation threshold may result from a progressive aqueous droplet interlinking and clustering process. The fact that microemulsion conductive behaviour at higher water contents can be described by the effective-medium theory equation (4), in which the values of Φ are related to those of Φ_w with the constant ratio 1.18 as determined at the percolation threshold, suggests that the clustering process does not affect the membrane-to-water phase volume proportion. This result appears to be an extension of that reported by Lagües and coworkers^{19,23} as to the constancy of (a/a_0) with Φ_w increasing up to 0.3 in water-in-cyclohexane microemulsions. Note that the present experiments yield an interesting idea as to the conduction mechanism in microemulsions. The fact that, p_s being fixed, σ increases with Φ while σ_1 remains constant proves that the conduction mechanism taking place in percolating microemulsions is essentially interfacial, as also suggested by Lagües *et al.*¹⁹. In support of this hypothesis, note that σ_1 , which was found to increase proportionally to the amount of surfactant, exhibits values that are lower than those of the corresponding KC1 aqueous solutions. For instance, the value of σ_1 , as deduced from Fig. 3, is 1.31 S m^{-1} while the aqueous solution with the same molar fraction of solubilised KC1 would display a conductivity of 4 S m^{-1} or so.

The droplet interlinking and clustering process suggested by the percolative behaviour of microemulsion conductivity might be predictive of the phase inversion phenomenon, the w/o to o/w transition taking place through the constitution of intermediary equilibrium states of the bicontinuous type¹⁸, a statistical model of which was set up by Talmon and Prager²⁵ who treated 'the microemulsion structure as a random geometry of interspersed oil and water domains generated by a Voronoi tessellation', the surface active agents being assumed to be entirely adsorbed at the water/oil interface.

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Primate facial allometry and interpretations of australopithecine variation

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Pilbeam and Gould¹ have discussed African Plio-Pleistocene hominid evolution in the context of allometry (size-dependent morphological change). These authors demonstrate that some general aspects of australopithecine morphology (tooth, brain and body size) support the hypothesis that certain early African hominids were merely scaled variations of each other at different sizes. They also speculate that the methods applied to these very broad anatomical categories can be extended to more specific and detailed traits, especially in the face and cranium. Such traits underlie most taxonomic and phylogenetic discussions of the early African Hominidae, so it is useful to follow Pilbeam and Gould's lead, as we do here, and investigate the structural differences in the australopithecine face and cranium in a quantifiable fashion.

We chose 16 standard measurements of the face and its relation to the neurocranium provided by Tobias² for the best preserved australopithecine specimens: *Australopithecus boisei*, represented by Olduvai Hominid 5 (OH 5), and *Australopithecus africanus*, represented by Sterkfontein 5 (Sts 5). The measurements have also been taken on other available australopithecine material (SK 48 and especially KNM-ER 406) although these are less complete and not as well described as the two crania that will be the focus of our analysis. The measurements are listed in Table 1.

Earlier allometric studies of primates^{3–12} have relied on bivariate comparisons of measurements. As it is difficult to synthesise the resulting patterns when multiple measurement combinations are involved, we use a multivariate method that allows the growth pattern to be represented as a coherent, covarying entity. A principal component model¹³ is available for this purpose but is better suited to intraspecific studies than to the interspecific approach which, like Pilbeam and Gould, we shall use. Thus, for the two fossils, a vector of allometric

coefficients (k) can be computed that connects the two points in the logarithmic hyperspace defined by the 16 dimensions¹⁴:

$$k_i = \frac{(\log x_{i1} - \log x_{i2})p^{1/2}}{[\sum_{j=1}^p (\log x_{j1} - \log x_{j2})^2]^{1/2}}$$

where p is the number of measurements and x the array of measurements. This two-dimensional solution is automatically of rank one. These coefficients, which describe the relative changes in proportions with increasing size in the two australopithecine morphs, are compared with the characteristic allometry between primate species. A somewhat novel sampling design is used for non-hominid primates. If all primates were to be treated as a single sample, inherent taxonomic differences which happen to coincide with size changes would be mistakenly identified as allometric in nature. For example, prosimians have quite elongated faces and snouts as well as generally small body size when compared with anthropoids, but this difference is not allometric. Therefore, we have constructed a sample of pairs of closely related but size-differentiated primate taxa, just as we are comparing australopithecine species. The statistical properties of pairwise allometric vectors can be compared with australopithecine values.

For each sampled species the modal-sized individual of five randomly selected male and five female specimens (or less depending on availability) was measured. Each complete pair of taxa yields four vectors, compared between the male and female of one to the two typical individuals of the other. The 12 species pairs result in a sample of 33 allometry vectors that in turn was visually selected from 200 specimens. The dimorphic species pairs are: *Indri indri*, *Avahi laniger*; *Cheirogaleus major*, *Microcebus murinus*; *Lemur variegatus*, *Lepilemur mustelinus*; *Galago crassicaudatus*, *Galago demidovii*; *Perodicticus potto*, *Loris tardigradus*; *Saguinus midas*, *Cebuella pygmaea*; *Ateles belzebuth*, *Saimiri sciureus*; *Nasalis larvatus*, *Presbytis melalophus*; *Erythrocebus patas*, *Cercopithecus talapoin*; *Papio cynocephalus*, *Macaca fascicularis*; *Hylobates syndactylus*, *Hylobates klossii*; *Pan gorilla*, *Pan troglodytes*. The sample pairs consists of four species of genera, six genera within subfamilies and two representatives of the large and small endpoints of families. The exact level of taxonomic distinctness between robust and gracile australopithecines remains controversial¹⁵⁻¹⁷, but is probably less than some of the intergroup differences in this comparative sample. Nevertheless, the variation that is non-allometric among the species pairs will be random and non-cumulative compared with that resulting from the consistent size discrepancy.

The allometries are shown in Table 1. Among primates in general, facial breadths are negatively allometric to superior facial length (that is large primates have disproportionately long faces); alveolar height is highly variable; nasal aperture width is positively allometric to its height, but nasal bone width is negative to length; neurocranial measurements are generally negatively allometric to facial measurements, and cranial lengths are positive to the breadths; the foramen magnum area, which is related to brain size¹⁸, has a relatively invariant relationship to other measurements and shows a value near the 0.66 scaling coefficient of brain to body which is characteristic of primates as a whole¹².

Between *A. africanus* (Sts 5) and *A. boisei* (OH 5), shape differences in facial height and the first four parameters of facial breadth co-vary closely with the interspecific primate allometries. This signifies that general primate growth tendencies do satisfactorily explain shape differences within the coronal plane of the face of australopithecines. The relatively narrow nose of the OH 5 specimen, on the other hand, is contrary to the primate trend, and the coefficient for nasal width falls outside the 99% confidence limits for the primate sample. The inferior tapering of the OH 5 nasal bones (characteristic also of other *boisei* and *robustus* specimens) is a unique phenomenon which is in no way accounted for by allometry. The only living primate in which a similar condition exists is the Asian colobine, *Rhinopithecus*. The longer nasal bones of OH 5, however, are again as

Table 1 Allometry coefficients comparing facial measurements of two species of *Australopithecus* with the means and s.d. values of primate interspecific allometry coefficients based on the same measurements

Measurement (endpoints)	Primates		
	OH 5-Sts 5	Means	(s.d.)
Inner biorbital breadth (fmo-fmo)	0.64	0.63	(0.19)
Bizygomatic breadth (zy-zy)	0.84	0.80	(0.15)
Maxillary breadth (zm-zm)	0.90	0.89	(0.20)
Anterior interorbital breadth (mf-mf)	1.08	1.07	(0.64)
Superior facial height (n-pr)	1.19	1.10	(0.26)
Cranial length (g-op)	0.49	0.72	(0.17)
Horizontal projective length (pr-i)	0.37	0.87	(0.14)
Superior facial length (ba-pr)	0.35	0.98	(0.14)
Bimastoid breadth (ms-ms)	0.80	0.61	(0.16)
Maximum biparietal breadth	0.39	0.53	(0.16)
Alveolar height (ns-pr)	0.80	0.39	(1.04)
Maximum nasal width	0.28	1.03	(0.20)
Nasal aperture height (ns-rh)	1.03	0.86	(0.33)
Nasal bone inferior breadth	-2.79	0.77	(0.27)
Nasal bone length (n-rh)	1.15	1.28	(0.56)
Foramen magnum (area ^{1/2})	-0.18	0.65	(0.11)

fmo, frontomale orbitale; zy zygon; zm, zygomaxillare; mf, maxillofrontale; n, nasion; pr, prosthion; g, glabella; op, opisthocranium; i, inion; ba, basion; ms, mastoidale; ns, nasospinale; rh, rhinion.

would be expected from the primate comparative sample. The australopithecine bimastoid (inferior) cranial breadth grows disproportionately relative to other cranial length and breadth measurements but still falls within two standard deviations of the primate mean. The two cranial breadths do not differ nearly as much between australopithecine and primate values as do the three lengths. The small allometry coefficient for horizontal projective length relates to the vertical face and lack of prognathism in OH 5, whereas larger primates are generally more prognathic. This measurement and the related superior length are significantly different in the two lists of coefficients. The average coefficient for facial measurements divided by the average of neurocranial dimensions is 1.75 between OH 5 and Sts 5, indicating the strong rate of allometric increase of face to calvaria. This figure is also positively allometric among primates at 1.26, but the latter value is significantly lower. The smaller foramen magnum of the *A. boisei* specimen also departs sharply from the expected primate value, although cranial capacity itself seems to scale according to the primate norm in all the australopithecines¹.

Thus, there are considerable parallels between the OH 5/Sts 5 australopithecine shape differences and those generally characterising larger and smaller related primates, especially within the face; and between the face and neurocranium. However, as previously noted, there are several departures from the primate vector in nasal aperture shape, the nature of the nasal bones, prognathism and foramen magnum area. When the relatively recently discovered East Rudolf 406 robust cranium is substituted for OH 5 in the calculations, identical results to these are obtained except for the larger foramen magnum of ER 406. Swartkrans 48 also yields highly similar allometry coefficients when compared with Sts 5, with the possible exception of alveolar height.

The more general measurements therefore support the hypothesis of Pilbeam and Gould¹ of australopithecine variation, whereas the more specific do not. With regard to the face and cranium, OH 5, KNM-ER 406 and SK 48 in many ways represent scaled-up versions of Sts 5. As the best estimates now indicate that Sts 5 is 0.5-1.0 Myr older than any of these other fossil crania¹⁹⁻²¹, our results supplement evidence²² for evolution of the robust australopithecines in South Africa from antecedent populations resembling *A. africanus* and the subsequent migration of the *A. boisei* stock to East Africa. Further investigation of Johanson and White's recently proposed model^{22,23} of early hominid evolution in Africa, using Pilbeam and Gould's allometry hypothesis¹, should include

documentation of divergent modes of change between crania of *A. afarensis* with its proposed descendent species *H. habilis* (encephalisation trend) and *A. africanus* (allometric trend).

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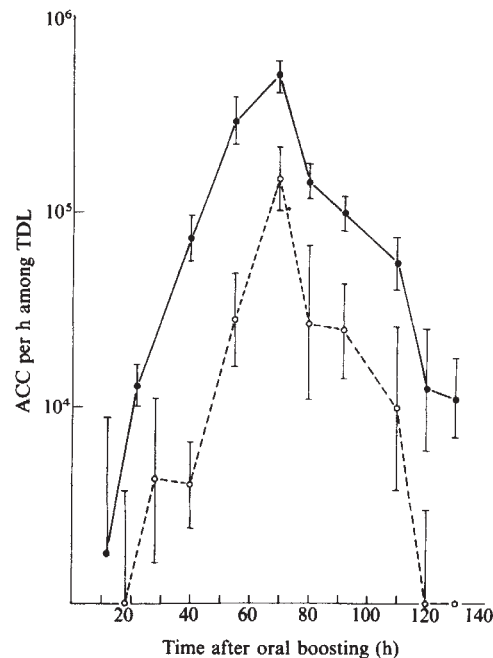


Fig. 1 Antitoxin-containing cells (ACC) among TDL from normal (●) and protein-deficient (○) rats after oral immunisation with CT. Wistar-Lewis rats were begun on test diets at age 7 weeks. For immunisation, CT was given with sodium bicarbonate by intragastric tube to conscious fasting rats. The priming dose was 40 mg crude toxoid (Wellcome) plus 1.5 mg crude toxin (NIH lot 001) given at age 11 weeks. The booster dose was 40 mg crude toxoid given 2 weeks later. These antigens are described elsewhere, as is evidence that this sequence provokes a vigorous mucosal antitoxin response in normal rats⁶. The thoracic duct was cannulated and antitoxin-containing cells among TDL were counted using a fluorescent antibody technique specific for cholera antitoxin⁴. Values are means $\pm$ s.e.m. for 3–9 rats given HP or LP diets.

Protein deprivation causes reversible impairment of mucosal immune response to cholera toxoid/toxin in rat gut

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Secretory antibodies may be the major defence against mucosal infections, especially those due to viruses and non-invasive pathogens such as *Vibrio cholerae* and toxinogenic *Escherichia coli*. The high incidence of mucosal infections in malnourished protein-deficient children¹ may result from defective antibody production², but evidence for this is conflicting³. We report here that protein deficiency markedly impairs the mucosal immune response to cholera toxoid/toxin (CT), a protein antigen, in rats and that this impairment is rapidly reversed by refeeding.

Protein deficiency was induced in 7-week-old Wistar-Lewis or Fischer rats weighing 140 g by feeding a low protein (3.2% casein) diet which was otherwise nutritionally complete (LP). The control diet was similar but contained 24% protein (HP). As rats given LP ate more for their weight than those given HP, the diet of LP rats was limiting only for protein. After 6 weeks of HP diet rats weighed 240 g whereas those given LP diets weighed 125 g (serum protein 69% of HP rats). LP rats had lymphoid atrophy but the intestinal mucosa was morphologically normal and they appeared well. Refeeding with HP after 6 weeks of LP diet produced rapid growth; within 2 weeks serum proteins were normal and 50% of the weight deficit was recovered.

Large thoracic duct lymphocytes (TDL), especially those containing IgA, arise from Peyer's patches and are direct precursors of IgA plasma cells in intestinal lamina propria^{4,5}. Table 1 shows the effect of protein deficiency on the number of TDL in unimmunised rats. Diminished numbers of large TDL, including those containing IgA, suggested an impaired mucosal immune response to enteric antigens. This was confirmed in rats immunised orally with CT⁶. HP rats showed numerous antitoxin-containing lymphoblasts among TDL after the second dose of CT; this response was reduced by 73% in rats fed LP 4 weeks before the first dose of CT (Fig. 1). In HP and LP rats more than 96% of antitoxin-containing cells among TDL contained IgA.

Table 1 Thoracic duct lymphocytes (TDL) in unimmunised normal and protein-deficient rats

	Total TDL	Large TDL	TDL containing IgA
HP diet	21 (1.1)	2.1 (1.2)	0.24 (1.3)
LP diet	9.0 (1.1)	0.61 (1.1)	0.13 (1.2)
<i>P</i>	<0.001	<0.001	0.06

Fischer rats were given test diets for 6 weeks starting at age 7 weeks. The thoracic duct was then cannulated for collection of TDL. Total TDL and large TDL were determined with a Coulter counter. The number of IgA-containing TDL was determined by autoradiography with ¹²⁵I-labelled rabbit F(ab')₂ anti-rat IgA⁴. Geometric mean cells h⁻¹ $\times 10^{-6}$ ($\pm$ s.e.m.), *n* = 10; *P* values by Student's *t* test.

The antitoxic response in ileal lamina propria was also impaired in LP rats. Feeding LP diet for only 4 weeks (2 weeks before the priming dose of CT) reduced the number of antitoxin-containing cells in ileal mucosa by more than 90%; however, an LP diet begun with priming was without effect (Fig. 2a). The mucosal response was also impaired by about 75% when LP rats were primed parenterally (Fig. 3), indicating that impairment was not due to poor absorption of the priming dose of oral CT. Likewise, the impaired response was unlikely to be due to poor absorption of the booster dose as it was at least 20 times the amount needed to produce a maximum response in previously primed normal rats (N.F.P., unpublished observations).

The possibility that refeeding with HP would correct the impaired mucosal immune response was also studied. Despite having had 6 weeks of LP diet, rats refed 2 weeks before starting enteric immunisation showed normal mucosal antitoxin responses; even when refeeding was begun with enteric priming, substantial recovery of responsiveness occurred (Fig. 2b).

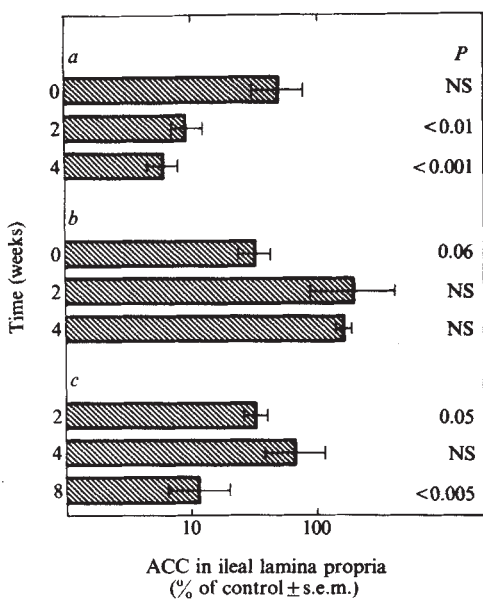


Fig. 2 Antitoxin-containing cells (ACC) in ileal lamina propria of protein-deprived and refed rats. Wistar-Lewis rats were started on LP diets at age 7 weeks. The oral priming and boosting doses of CT are described in Fig. 1 legend. *a*, Time indicates period of LP diet before priming and the LP diet was continued until rats were killed; oral boosting was 2 weeks after priming. *b*, Time indicates period of refeeding before priming. The LP diet was given for 6 weeks, after which HP diet was given. The time of oral priming after refeeding is indicated; oral boosting was 2 weeks after priming. *c*, Time indicates period of LP diet before priming. Oral priming occurred at the indicated interval after starting the LP diet; the diet was continued for 2 weeks after priming; refeeding with HP was then begun and oral boosting was 2 weeks later (4 weeks after oral priming). Scoring of ileal ACC was always 5 days after boosting. Controls were identically immunised and concurrently studied rats given HP diet only. Control responses averaged about 9,000 ACC mm⁻³. Each group contained 7–17 rats; *P* values were by the *t* test comparing LP rats with concurrently studied controls.

The selective effect of the LP diet on the primary component of the mucosal IgA response was studied by refeeding after enteric priming. Feeding LP for 4 or 6 weeks (2 or 4 weeks before priming), but refeeding with HP 2 weeks before boosting gave a near-normal mucosal antitoxin response; however, extension of the period of LP diet to 8 weeks before priming produced substantial impairment despite refeeding before boosting (Fig. 2c).

Thus the ability to mount a specific mucosal IgA response was impaired and corrected by relatively brief periods of protein restriction and refeeding, respectively. The mechanism by which protein deficiency impaired the IgA antitoxin response is unknown and is probably complex. Fewer antitoxin-containing cells among TDL in LP rats indicates that protein deficiency impaired the generation of specific IgA immunoblasts in Peyer's patches⁵; this effect was not specific for antitoxin as non-immune rats had fewer IgA-containing TDL. The greater impairment of the antitoxin response in ileal mucosa (Fig. 2a) than among TDL (Fig. 1) suggests that antigen-driven division of immunoblasts in the ileal mucosa⁵ was also impaired. Protein deficiency appeared to impair both primary and secondary components of the

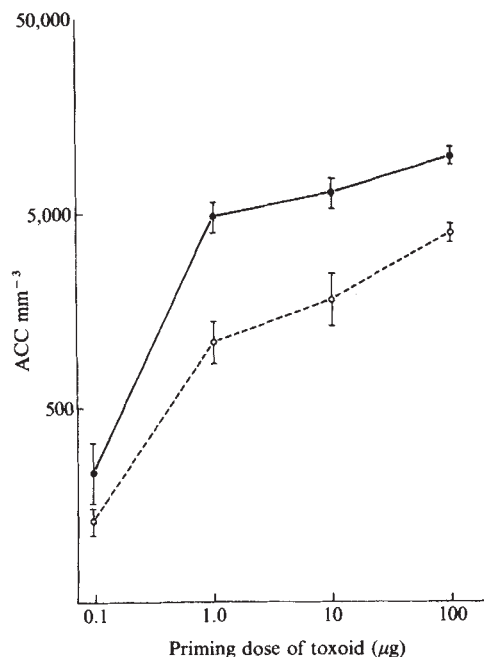


Fig. 3 Effect of protein deficiency on the enteric antitoxin response of rats primed intraperitoneally (i.p.) with purified toxoid. Seven-week-old Wistar-Lewis rats were fed HP (●) or LP (○) diets. Primary immunisation was with purified toxoid (Wellcome) given i.p. (0.1 ml toxoid plus 0.1 ml Freund's complete adjuvant) at age 11 weeks. Intragastric boosting with 40 mg crude toxoid was 2 weeks later. ACC in ileal lamina propria were scored 5 days after boosting; results are geometric mean $\pm$ s.e.m., *n* = 5–8 for each value.

mucosal immune response; there was direct evidence for an effect on priming (Fig. 2c); an effect on boosting is inferred from the shorter period of LP diet required to impair responses in rats not refed before boosting (Fig. 2a). The rapid recovery of responsiveness with refeeding parallels the previously described rapid regeneration of atrophic lymphoid tissue when protein-deficient mice are refed⁷.

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Extra-pituitary inhibition of testicular function by luteinising hormone releasing hormone

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Luteinising hormone releasing hormone (LHRH), produced by the hypothalamus, stimulates gonadotropin production by the pituitary¹⁻³. Paradoxically, chronic treatment with large doses of LHRH or its agonists causes a decline in testicular steroidogenesis and testicular LH/human chorionic gonadotropin (hCG) receptors; they also cause a decrease in the weight of testes, seminal vesicles and ventral prostate⁴⁻⁷. There are three possible mechanisms for the inhibitory action of LHRH and its agonists. At the pituitary level, prolonged action of LHRH or its agonists may make the pituitary refractory to further stimulation by LHRH, with a decrease in serum gonadotropins and subsequent atrophy of the male reproductive organs^{8,9}. It is also possible that pharmacological doses of LHRH stimulate the release of a large amount of LH, which in turn 'desensitises' the testis to further LH¹⁰⁻¹⁴. We have tested a third possibility, namely that LHRH and its agonists act directly on the testis. We report here that LHRH and LHRH agonists cause a decrease in testicular LH/hCG receptors and inhibit testicular steroidogenesis in hypophysectomised male rats, indicating a direct effect on the Leydig cells. This is consistent with our demonstration that LHRH and its agonists act independently of the pituitary to inhibit ovarian steroidogenesis in cultured rat granulosa cells and in hypophysectomised female rats *in vivo*¹⁵.

Immature hypophysectomised male rats were injected daily for 5 d with ovine follicle stimulating hormone (FSH) and/or LHRH analogues or the appropriate vehicle. After hypophysectomy, FSH reduces the general testicular regression and enhances the LH/hCG stimulation of testosterone production¹⁶⁻¹⁹. Following hormone treatment, animals were killed and testes were removed, decapsulated, weighed and their LH/hCG receptor content and steroidogenic potential determined.

LHRH analogues decreased the testicular weight in both FSH-treated and untreated hypophysectomised rats (Fig. 1a). The testes of FSH-treated animals weighed significantly more than the testes of untreated hypophysectomised rats: 139.0 ± 5.1 mg compared with 72.6 ± 1.5 mg (mean $\pm$ s.e.m.). Daily injections of [D-Leu⁶(N^oMe)Leu⁷]LHRH (analogue I, 200 μ g per d) or des-Gly¹⁰-[D-Leu⁶(N^oMe)Leu⁷,Pro⁹-NHet]LHRH (analogue II, 50 μ g per d) decreased the testis weight of FSH-treated animals by 12 and 21%, respectively ($P < 0.01$). In untreated hypophysectomised rats, analogue II caused a 20% decrease in testis weight (72.6 ± 1.5 mg to 57.4 ± 2.5 mg; $P < 0.01$).

Treatment with LHRH analogues reduced the binding of ¹²⁵I-hCG to Leydig cells in both FSH-treated and untreated hypophysectomised rats (Fig. 1b); binding capacity was 2.1 times greater in the former than the latter. In untreated hypophysectomised rats, analogue II caused a significant decrease in hCG binding ($P < 0.01$). Likewise, LHRH analogues I or II given together with FSH significantly decreased FSH-enhanced hCG binding in testicular Leydig cells by 66% and 84%, respectively. Indeed, the effect of analogue II was so great that the testicular LH/hCG receptor content in animals treated with analogue II plus FSH was less than that in animals not treated with FSH ($P < 0.01$). Furthermore, LHRH at 50 μ g per d also inhibited FSH-enhanced hCG binding by 40%, from 4.21 ± 0.45 ng per testis to 2.51 ± 0.05 ng per testis. The inhibitory effect of LHRH and its analogues was also apparent when hCG

binding was expressed as pg hCG bound per mg testis: control, 27.5 ± 1.4 ; analogue II, 9.6 ± 0.6 ; FSH-treated, 30.3 ± 3.2 ; FSH+analogue I, 11.7 ± 1.5 ; FSH+analogue II, 6.3 ± 0.6 ; FSH+LHRH, 16.5 ± 0.3 .

LHRH analogues also inhibited stimulation of testicular steroidogenesis by hCG. Decapsulated testes were incubated for 3 h at 34 °C in polyethylene vials, half of which contained a saturating concentration of hCG (500 ng ml⁻¹). The androgen concentration in the medium was then determined by testosterone radioimmunoassay²¹. The basal steroidogenic response was low in the control group, and was increased by FSH treatment for 5 d (Fig. 2). In contrast, concomitant administration of analogues I or II together with FSH significantly decreased basal steroidogenesis ($P < 0.01$). In the presence of a saturating concentration of hCG, testicular steroidogenesis was stimulated in all groups (Fig. 2, cross-hatched bars). Pretreatment with FSH considerably enhanced the steroidogenic responsiveness of

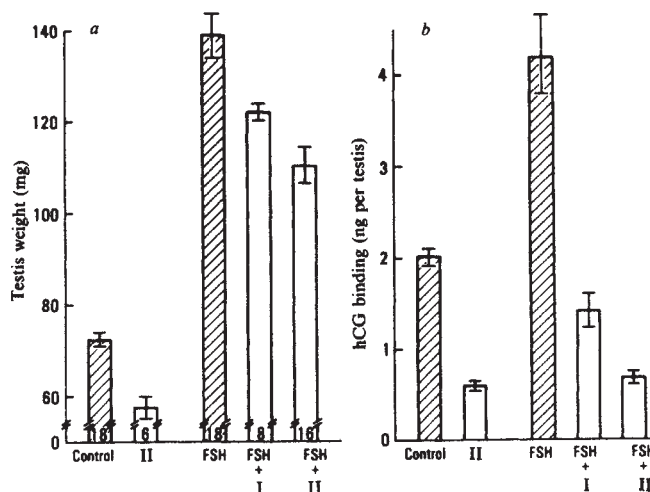


Fig. 1 Effect of treatment with FSH and/or LHRH analogue on testicular weight and ¹²⁵I-hCG binding capacity of immature (21–23-d-old) hypophysectomised male rats (Hormone Assay Labs). The completeness of hypophysectomy was determined by two parameters. First, 9 d after hypophysectomy of 21-d-old male rats, considerable testicular involution was observed with reduction of testis weight from 169 ± 13 mg to 72.6 ± 1.5 mg. Intact 30-d-old rats have a mean testis weight of 469.4 ± 12 mg. Second, serum LH was measured in hypophysectomised male rats treated with 200 μ g LHRH analogue I ([D-Leu⁶(N^oMe)Leu⁷]LHRH) or 50 μ g analogue II (des-Gly¹⁰-[D-Leu⁶(N^oMe)Leu⁷,Pro⁹-NHet]LHRH) for 0, 1, 3 and 6 h. In all samples tested, LH in serum was below the limit of detection by LH radioimmunoassay (less than 5 ng NIH-LH-RP-1 per ml). **a**, Beginning 4 days after hypophysectomy, rats received subcutaneous injections daily for 5 d. They received an injection of 50 μ g ovine FSH (NIH-FSH-S12) dissolved in 0.1 ml medium 199 with or without an injection of LHRH analogue (200 μ g analogue I or 50 μ g analogue II) dissolved in 0.1 ml corn oil. Control animals received vehicle alone. Between 20 and 24 h after the last injection, animals were decapitated and their testes were removed, decapsulated and weighed (mean $\pm$ s.e.m.). The numbers inside the column indicate the number of testes used. **b**, Decapsulated testes were homogenised in a Kontes ground-glass tissue grinder in ice-cold phosphate buffer (0.01 M, pH 7.4) containing 0.15 M NaCl and 0.1% bovine serum albumin (BSA). After centrifugation at 20,000g for 15 min, the crude membrane pellet was resuspended to a final concentration of 1 testis per 1.5–3 ml buffer. The concentration of LH/hCG receptors was determined by incubation of serial dilutions of resuspended pellet with a saturating concentration of labelled hCG. Excess unlabelled hCG (100 IU Pregnyl) was added to half of the incubation tubes to obtain nonspecific binding to testis membranes. ¹²⁵I-hCG (CR-119) was prepared by radioiodination with chloramine T as described previously²⁰. The specific activities of the labelled hCG were determined by self-displacement analysis in the radioligand–receptor assay²⁸ and varied from 20,000 to 80,000 c.p.m. per ng. The fraction of the ¹²⁵I-hCG able to react specifically with an excess of receptor (that is, the maximum binding activity) was determined for each tracer preparation. This value (45–65%) was used as a correction factor during quantitative analysis of LH/hCG receptor²⁸. After incubation of the testicular membrane fraction with ¹²⁵I-hCG at room temperature (22–24 °C) for 16–18 h, the mixtures were diluted with 3 ml ice-cold buffer and centrifuged at 1,500g for 15 min. Pellets were washed once with buffer and then counted in a γ -spectrometer. After correcting for the radioactivity bound to equal aliquots of samples incubated in the presence of an excess of unlabelled hormone, specifically bound radioactive hormone was determined as ng hCG bound per testis. Data represent the mean $\pm$ s.e.m. of three experiments.

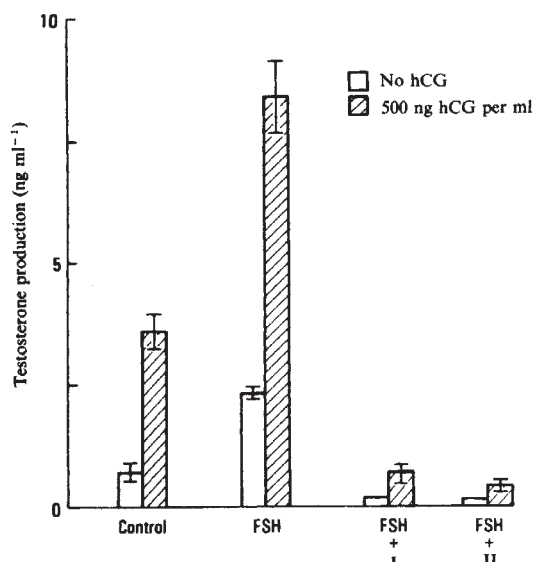


Fig. 2 Effect of treatment with FSH and/or LHRH analogue (I or II) on the steroidogenic capacity of rat testes *in vitro*. Hypophysectomised immature male rats were treated with FSH and/or LHRH analogue as described in Fig. 1. Testes were decapsulated, washed once in ice-cold saline and transferred to polyethylene vials containing 2.0 ml ice-cold medium 199 with 0.1% BSA and 0.1 mM 1-methyl-3-isobutyl xanthine (Aldrich Chemical). Each vial contained one testis, either with or without saturating concentrations of hCG (1,000 ng). All vials were then incubated for 3 h at 34 °C under 95% air, 5% CO₂. Androgen concentration in the incubation medium was measured by specific radioimmunoassay using testosterone antiserum²¹. The antiserum shows no cross-reaction with C₂₁ or C₁₈ steroids but cross-reacts 100% with dihydrotestosterone, 80% with androstenedione, 30% with 5 α -androstenediol and 4% with androsterone. The latter two steroids contribute significantly to total androgen production in immature rats. Three to five samples were used for each point and duplicate determinations were carried out by radioimmunoassay. The results are expressed as mean $\pm$ s.e.m.

testes to hCG, whereas a smaller increase was found in untreated hypophysectomised rats. In contrast, concomitant treatment with LHRH analogues I or II markedly depressed the ability of FSH to increase testicular steroidogenic responsiveness to hCG. hCG-induced androgen production in animals treated with FSH plus LHRH analogue is lower than that of control animals receiving no FSH.

These observations indicate that LHRH agonists, in addition to their releasing action at the pituitary gonadotrophs, directly inhibit testicular function. These results may help explain the potent anti-reproductive effect of high doses of LHRH agonists in intact male animals⁴⁻⁷. LHRH agonists induce a substantial decrease in LH/hCG receptors as well as decreasing the steroidogenic responsiveness of Leydig cells (Figs 1 and 2). This may, in turn, result in degenerative changes in seminiferous tubules (spermatogenesis), ventral prostate and seminal vesicles^{4-7,22}. Although the testis is the most probable site of action of the LHRH agonists, the use of the hypophysectomised male rats in our study does not exclude the possibility that LHRH agonists act on other organs such as the adrenal, resulting in secondary inhibition of the Leydig cells.

The mechanism by which LHRH agonists inhibit Leydig cell function is unknown. Although LHRH binding sites have been identified in the anterior pituitary²³⁻²⁶, the tissue specificity of the binding has not been demonstrated conclusively. Indeed, Heber *et al.*²⁷ reported the presence of specific high capacity, low affinity LHRH binding sites in the testis. It is possible that LHRH agonists exert their inhibitory effect through gonadal LHRH receptors. In conclusion, our study provides the first evidence demonstrating an extra-pituitary inhibitory action of LHRH and LHRH agonists on testicular functions in hypophysectomised animals.

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Identified neurones isolated from leech CNS make selective connections in culture

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Neurones cultured *in vitro* offer distinct advantages for studying how processes grow towards their targets and form synaptic connections. In contrast to the complex events occurring during the development of the nervous system, synapse formation in culture can be analysed in a few neurones at a time and under controlled conditions¹. We have now dissected out and cultured single identified neurones from the central nervous system (CNS) of the adult leech. Various types of sensory cells, motor cells, and interneurons can be identified in leech ganglia—each with a stereotyped set of properties, including: (1) the electrical characteristics of its membrane, (2) the arborisation of its branches and the morphology of its terminals and (3) the pattern of connections it makes with other identified neurones, skin or muscle². Thus, cultured cells can be compared in detail with their counterparts *in situ*. We have found that isolated cells survive for several weeks, maintain their membrane properties, sprout and form selective connections.

Leech ganglia were dissected under sterile conditions and placed in culture medium consisting of Leibowitz L-15 and 2% fetal calf serum together with glucose 6 mg ml⁻¹ and gentamycin 0.1 mg ml⁻¹. The connective tissue capsule was cut (Fig. 1), allowing the neuronal cell bodies to pop out of the large glial cell that normally surrounds them³. For identification, the cells

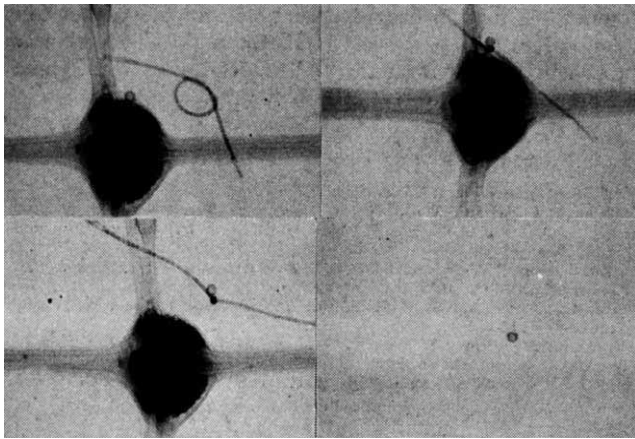


Fig. 1 Steps in the removal of an individual N (nociceptive) cell from a leech ganglion. The diameter of the N cell is about 60 μm .

were impaled with microelectrodes. However, this was not necessary for certain neurones which are unequivocally recognisable by their sizes and positions. Such cells include those used predominantly for the present experiments: the sensory cells that respond to pressure (P cells) and the large Retzius cells that cause mucus secretion. To isolate an identified cell a loop of fine nylon monofilament 13 μm in diameter was tied around its neck, pulled tight and drawn away from the ganglion (Fig. 1a). A few minutes later, after the cell had become sealed at the knot, it was freed by gently shaking the thread and transferred to a plastic tissue culture dish, coated with polylysine and collagen and containing approximately 2 ml of culture medium. Dishes were maintained at 20 °C, often without changing medium for 2 weeks.

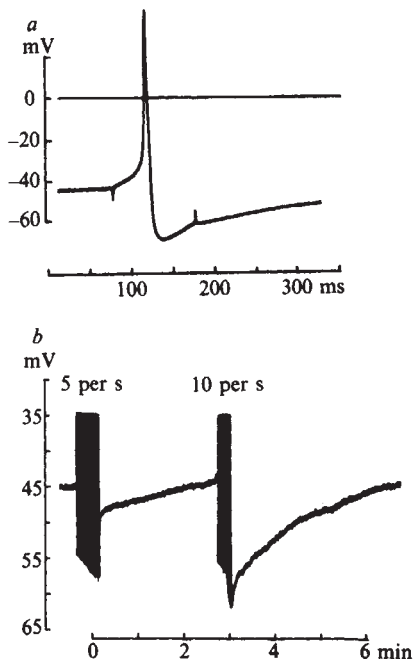


Fig. 2 *a*, Action potential from an N cell isolated for 21 days. The shape and undershoot are identical to those seen in N cells within an intact ganglion. *b*, Afterhyperpolarisation of a P sensory cell isolated for 24 days. Stimuli applied through the microelectrode produced impulses at 5 and 10 per s followed by a prolonged increase in membrane potential. In normal ganglia similar hyperpolarisation is caused by the activity of the Na-K pump and by an increase in K conductance.

For several weeks after removal from the ganglion, individual cells showed normal resting and action potentials. Figure 2a shows the action potential of a nociceptive (N) cell which had been kept isolated for 21 days. The shape of its action potential was indistinguishable from that of a normal N cell *in situ* and unlike that of any other type of cell in the ganglion². Similarly, other cells, such as touch cells, pressure cells, and Retzius cells could also be identified unambiguously by electrical criteria and in particular by their characteristic action potentials. These results suggested that the intracellular concentrations of ions and membrane permeabilities were not grossly changed in culture. Further evidence was provided by stimulating cells repetitively. Fig. 2b shows the hyperpolarization following trains of impulses in a P sensory cell. The hyperpolarisation was comparable to that observed in normal P cells *in situ*, where it has been shown to arise from a coupled sodium pump and a calcium-dependent increase in potassium conductance⁴.

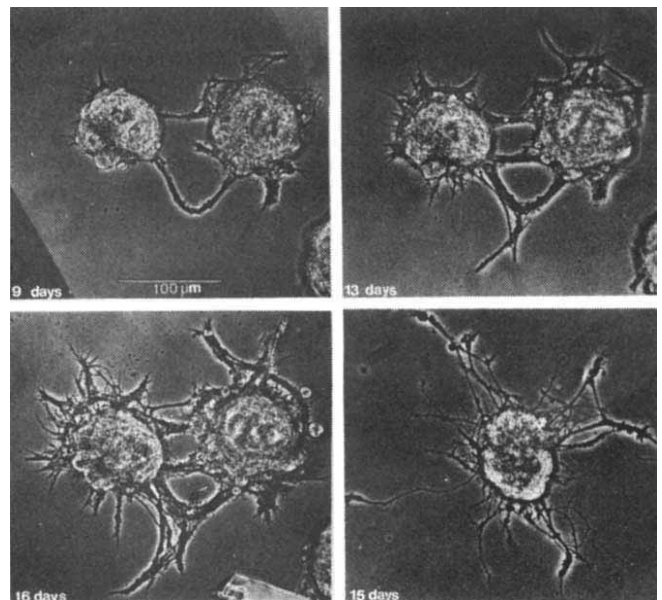


Fig. 3 Development of connections between a pair of Retzius cells over 16 days. Note the preferential distribution of processes running between the two cells and the relative paucity of processes running in other directions. The single neurone (a P cell) cultured on its own, shows more radial distribution of fibres.

After 5 to 7 days in culture, isolated cells began to sprout. Although leech neurones are monopolar *in vivo*, in culture a number of processes extended from the soma (Fig. 3). These processes had typical, filopodia-tipped growth cones, and tended to grow along each other, producing thickened, braided fascicles. Figure 3 shows the growth and development of neurites between two Retzius cells that were placed near one another. In this and other preparations studied over comparable times, bundles of processes were frequently observed passing between the cells, rather than at random. Single cells cultured alone sprouted, but usually without an obvious preferred direction (Fig. 3).

Cultured Retzius cells became electrically coupled and formed chemical synapses. The paired Retzius cells in each ganglion are electrically coupled to each other. When individual Retzius cells were dissected out and placed close to one another as in Fig. 3, or else in contact, they again became electrically coupled: current injected into one cell spread to its neighbour (Fig. 5). As in the animal, current passed equally well in both directions and considerable variation was seen in the degree of coupling, ranging from a minimal spread of current to nearly 1 : 1 coupling.

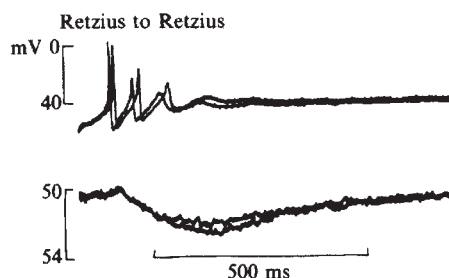


Fig. 4 Chemically mediated inhibitory potentials initiated at 22 days in one Retzius cell (lower trace) by impulses in its neighbour (upper trace). The electrical coupling between these cells was weak (see text). Impulses were initiated at the end of hyperpolarising pulse (not shown).

While the development of coupling has not been studied in detail, in most cultures of closely opposed cells coupling first became obvious by about 10 days.

Retzius cells also formed chemical inhibitory synapses upon each other in culture. Figure 4 shows an example from a pair of Retzius cells in which impulses in one cell gave rise to hyperpolarizing potentials in the other with a variable latency. Such potentials were not obvious in cases where the degree of electrical coupling was strong and where the electronically conducted potential tended to obscure events occurring shortly after the spike. For similar reasons it is hard to test for inhibitory connections of this type between Retzius cells within the CNS. Towards the head of the leech, however, ganglia are occasionally found in which the Retzius cells are coupled only weakly and there it may be possible to demonstrate inhibitory connections if they are normally present.

A long-term goal of these experiments is to examine the specificity of connections formed between identified neurones in culture. So far we have obtained one clear example of preferential connections. Retzius cells were paired either with another Retzius cell or with a different neurone, the sensory P cell. In the ganglion the P cells are not electrically coupled to Retzius cells⁶. In five dishes kept from 12 to 24 days, 17 out of 24 pairs of Retzius cells became coupled. In contrast, none out of 22 Retzius-P pairs and none out of 8 P-P pairs became coupled (Fig. 5). In the leech, regenerating neurones reform their connections with a high degree of precision⁵. However, novel connections are occasionally found after lesions have been made to the CNS⁷, and when ganglia are isolated in culture⁵. A similar relaxation of specificity may occur when individual cells are isolated and it would not be surprising if novel connections

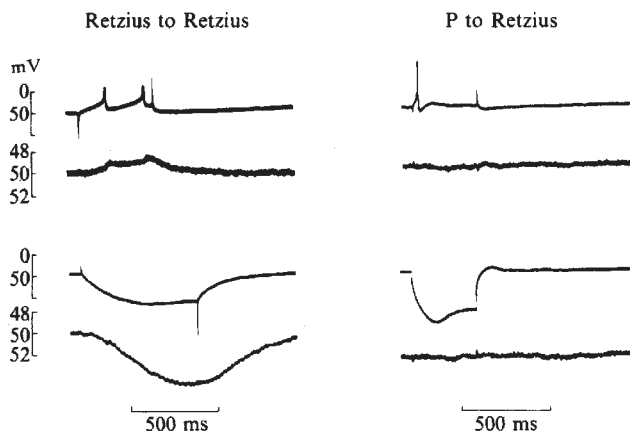


Fig. 5 Specificity of coupling at 24 days between paired Retzius cells (left) and paired Retzius P cells (right). Current spread from Retzius cell to Retzius cell but not from P cell to Retzius cell or vice versa. In this experiment the pairs of cells were in close apposition to one another in the same dish.

developed between cultured cells. For example, the inhibitory connections between Retzius cells may fall into this category.

We conclude that adult leech neurones can survive well in culture without the large glial cells that normally surround them and can form certain specific electrical connections. The selectivity of connections formed under these conditions presumably depends on inherent properties of the cells rather than on factors such as timing, competition or the availability of sites for synapse formation on the target cell. Given the wide variety of identified cells available in the leech ganglion such experiments could provide a basis for examining in greater detail the preferences that a cell exhibits in relation to a number of different potential target cells.

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A physiological correlate of disuse-induced sprouting at the neuromuscular junction

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Recent investigations have established that many of the normal properties of muscle fibres are maintained, at least in part, by muscle activity¹. Thus, a fall in resting membrane potential^{2,3}, an increase in input resistance⁴, and spread of acetylcholine receptors to extrajunctional sites⁴⁻⁶ can all be induced by abolishing muscle activity and prevented by direct stimulation of denervated muscle fibres^{7,8}. Muscle activity also exerts a trophic influence on the innervating motoneurons^{9,10}; furthermore it may be a factor in the regulation of sprouting^{11,12}. Brown and Ironton¹¹ found fine, "ultra-terminal sprouts" emanating from the endplates of muscles rendered inactive by chronic conduction block of the muscle nerve. Pestronk and Drachman¹² saw increased branching of the motor nerve terminal and a consequent increase in endplate size in similar conditions. If these sprouts at the endplates of inactive muscles were functional, one might expect more transmitter to be released in response to nerve stimulation¹³. We report here that both quantum content and spontaneous miniature endplate potential (m.e.p.p.) frequency are increased at the terminals of inactive (disused) muscles.

To effect disuse we used silicone cuffs impregnated with tetrodotoxin (TTX)⁶. TTX (1 mg) buffered with citric acid-sodium citrate (Sankyo) and 20 mg NaCl were dissolved in water; the solution was lyophilised and then mixed with 1.2 ml Silastic 382 (Dow Corning). Polymerising agent was added and the mixture was put in a glass tube (internal diameter, 4 mm) and hollowed out with a 17 gauge hypodermic needle. Female (except one) Sprague-Dawley rats (150-310 g) were anaesthetised with ether. The right or left sciatic nerve (experiments alternated sides) was exposed in the mid-thigh region, and a

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2–4-mm segment of the silicone cylinder was slit longitudinally and placed around the nerve. The efficacy of the block was assessed by checking the toe-spreading reflex which is invariably present when a normal animal is suspended by the tail; its absence is assumed to result from conduction block of the sciatic nerve⁶. The initial cuff abolished toe-spreading for 3–7 days. Rats were checked twice daily and a new cuff was implanted as soon as the reflex recovered. In this manner conduction block was maintained for a period of 8–9 days. Second cuffs were not usually as efficacious as the original, and sometimes as many as three changes were required. In some animals control cuffs, prepared as described above (including NaCl and citrate buffer) but without TTX, were implanted around the right or left sciatic nerve and left in place for 8–9 days. The toe-spreading reflex was not affected by the control cuff.

If conduction were blocked for 8–9 days, the animal was anaesthetised with Nembutal, and the soleus muscle, together with a length of muscle nerve, was excised from each leg. The muscles were pinned side by side in a lucite chamber superfused at room temperature by a solution of (mM): NaCl 136.8; KCl 5.0; CaCl₂ 1.0; MgCl₂ 8.0; NaH₂PO₄ 1.0; NaHCO₃ 15.0, through which 95% O₂ plus 5% CO₂ was continuously bubbled. Neostigmine methylsulphate (10⁻⁶ M) was included in the solution to enhance the m.e.p.s. Muscle fibres were impaled with conventional microelectrodes filled with 2.6 M KCl. Those fibres with a resting membrane potential of greater than 55 mV in which m.e.p.s could be distinguished clearly were selected for determination of quantum content. On stimulating the muscle nerve at a frequency of 0.5 s⁻¹, ~200 endplate potentials (e.p.s), then approximately 50 s of spontaneous m.e.p.s were recorded. Recordings were taken alternately from disused and control muscles. Quantum content (m) was estimated by the method of failures¹⁴ except for a few endplates where no failures occurred and m was calculated from the coefficient of variation of the e.p.s. amplitude distribution¹⁴. When both methods were applied for purposes of comparison (12 endplates), the m values agreed to within 15% of the average.

Table 1 Quantum contents at disused and control endplates

Expt	M_{TTX}	M_{con}	Ratio ($M_{\text{TTX}}/M_{\text{con}}$)
1	2.3	1.8	1.3
2	2.9	1.8	1.6
3	3.3	1.8	1.8
4	3.4	3.5	1.0
5	4.9	4.2	1.2
6	3.2	2.4	1.3
7	2.2	1.4	1.6

M_{TTX} is the mean m of soleus endplates on the side where conduction of the sciatic nerve was chronically (8–9 days) blocked with TTX cuffs. M_{con} is the mean m of control endplates on the contralateral side. At least five fibres were sampled on each side.

Table 1 shows results obtained from seven animals. M_{TTX} represents the mean of m values determined at 5–7 soleus endplates on the disused side. M_{con} is the similarly determined mean from the contralateral control soleus of the same animal. The mean m of junctions from disused muscles was greater than that on the control side in six out of seven experiments, the average difference being 40%. These data were analysed with the two-tailed t test for matched pairs. The difference in mean quantum content between the disused and control sides was highly significant ($0.005 < P < 0.01$), and remained significant even when the two highest ratios ($M_{\text{TTX}}/M_{\text{con}}$) were excluded (expts 2, 3).

Table 2 presents data from five control animals, M_{cuff} being the mean m value of soleus endplates from the leg in which a control cuff (see above) had been placed. M_{con} is again from the contralateral control soleus. No significant difference in mean quantum content between the two sides was noted ($0.20 < P < 0.30$).

Table 2 Quantum contents from control animals

Expt	M_{cuff}	M_{con}	Ratio ($M_{\text{cuff}}/M_{\text{con}}$)
1	1.5	1.5	1.0
2	1.7	2.2	0.8
3	1.3	1.3	1.0
4	2.5	2.4	1.0
5	1.3	1.6	0.8

M_{cuff} is the mean m of soleus endplates from the leg in which a control cuff had been placed. M_{con} is the mean m from the contralateral soleus.

At the amphibian neuromuscular junction, quantum content and spontaneous m.e.p.p. frequency (f) are positively correlated with endplate size¹³. If the terminal area is enlarged by sprouting, then one might expect f to increase as well as m . We made determinations of f from at least four disused and four control fibres in each of five experiments. After 8 days of disuse, f at junctions from disused muscles was higher than at contralateral control junctions in all five preparations. The mean values were respectively $1.2 \pm 1.0 \text{ s}^{-1}$ and $0.7 \pm 0.4 \text{ s}^{-1}$. With the matched pairs t test, f on the disused side was significantly higher ($0.02 < P < 0.05$). Animals prepared with control cuffs showed no significant differences in f between the cuff and contralateral sides. However, as was the case at soleus endplates in the mouse (S. T. Carbonetto and J. N. Weakly, personal communication), f and m were not significantly correlated in our data. One explanation may be that f and m vary less at mammalian than at amphibian endplates. For example, the coefficient of variation of m values from our control rat solei is 0.5, whereas in frog sartorius it is 1.2 (M. Kuno, S. A. Turkianis and J. N. Weakly, personal communication). The variability of f values from the two preparations is also quite different.

One might argue that the presence of the cuff could itself result in degeneration of some motor axons, and that partial denervation rather than disuse might account for these results. However, application of control cuffs did not result in any significant change in quantum content on the cuff side. Moreover, we did not observe fibrillation in any preparation, implying that most or all motor axons remained intact. Disuse itself has been reported to cause fibrillation^{3,4}, but work in the cat^{9,15} agrees with our finding of none. We have no explanation for this discrepancy.

The present results indicate that at the neuromuscular junctions of chronically disused muscles, presynaptic terminals release increased amounts of transmitter in response to nerve stimulation and exhibit increased spontaneous release of acetylcholine quanta. These observations are consistent with the hypothesis that chronic disuse enlarges the functional endplate area, possibly as a result of increased terminal fibre branching and endplate length reported by Pestronk *et al.*¹². Lømo and Rosenthal⁴ have suggested that quantum content may be increased at the junctions of disused soleus muscles (slow twitch) but not those of disused extensor digitorum longus muscles (fast twitch). In the mouse, soleus endplates sprout more vigorously than peroneus tertius (fast twitch) endplates in response to disuse¹¹. These findings suggest that fast and slow twitch muscles may differ in their ability to produce the signal for sprouting, or that there may be differences in the ability of their motor terminals to respond.

Disuse may affect transmitter release in other ways; for example, the membrane properties of presynaptic terminals may change, thereby affecting quantum content. While we cannot exclude this possibility, it is not clear how such changes in membrane properties would also increase spontaneous m.e.p.p. frequency. With respect to the signal that may trigger this increase in quantum content, it remains uncertain whether muscle inactivity or the disused terminals themselves are responsible. It is interesting in this regard that Gallego *et al.*¹⁵ have reported an increase in synaptic efficacy at the disused Ia synapses of spinal motoneurons.

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GABA may be a neurotransmitter in the vertebrate peripheral nervous system

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γ -Aminobutyric acid (GABA) is an inhibitory neurotransmitter in the peripheral nervous system of certain invertebrates and is thought to be a major transmitter in the vertebrate central nervous system^{1–3}. In this report we present evidence that GABA may also be a neurotransmitter in the vertebrate peripheral autonomic nervous system. We have used light and electron microscopic autoradiography to analyse high-affinity uptake of ³H-GABA into the myenteric plexus of the guinea pig taenia coli, both *in situ* and in a tissue culture preparation. In the isolated myenteric plexus, we have measured the specific activity of glutamic acid decarboxylase (GAD; EC 4.1.1.15), the enzyme responsible for conversion of glutamic acid to GABA in GABAergic neurones^{4,5}, and assessed the ability of this tissue to accumulate ³H-GABA newly synthesised from ³H-glutamic acid. Furthermore, we have measured the levels of endogenous GABA in strips of taenia coli containing the myenteric plexus.

For autoradiography, myenteric plexus from 1–8-day-old guinea pigs grown *in vitro* as described previously⁶ was incubated in Krebs solution containing 2×10^{-8} M ³H-GABA ($1 \mu\text{Ci ml}^{-1}$, 66 Ci mmole⁻¹; [2,3-³H]GABA, Radiochemical Centre) and 10^{-3} M β -alanine to block non-neuronal uptake of GABA⁷. After 20 min incubation at 20 °C, the samples were washed with Krebs solution, fixed in 4% glutaraldehyde in Krebs solution, washed with distilled water, air dried, dipped in Ilford L-4 or Kodak NTB2 emulsion and developed with Agfa Rhodinal developer after exposure for 10–14 days. Examination by light microscopy showed that although the density of silver grains was low over the non-neuronal cells and almost totally absent from most of the neurones, some neurones in the cultures were heavily labelled with grains (Figs 1, 2). Labelling was restricted to small ($\sim 16 \times 17 \mu\text{m}$) to medium-sized neurones, the large neurones ($\sim 40 \times 27 \mu\text{m}$) always being unlabelled. The grains seemed evenly distributed over the

nucleus, cytoplasm and neuronal processes. The cell bodies were of various shapes and the labelled axons emanating from them could often be traced over long distances in the cultures, in some cases running together with unlabelled axons in thick axon bundles, but in others lying singly within the explants or in the outgrowth zones around them.

The heavy labelling was totally absent if 10^{-3} M *cis*-1,3-aminocyclohexane carboxylic acid (ACHC), an inhibitor of neuronal GABA uptake⁸, was included in the incubation medium, thus confirming the morphological identification of the labelled cells as neurones. If the cultures were incubated without β -alanine but in the presence of 10^{-3} M ACHC a few cells within the explant were moderately labelled. This labelling was blocked by 10^{-3} M β -alanine and probably occurred over glial cells. Explant cultures of rat thoracic sympathetic ganglia showed no neuronal autoradiographic labelling in any of the above conditions.

These findings were extended by electron microscopic autoradiography of the myenteric plexus *in situ*. Taenia coli containing the myenteric plexus were dissected from 1-day to 3-month old guinea pigs and incubated with ³H-GABA as

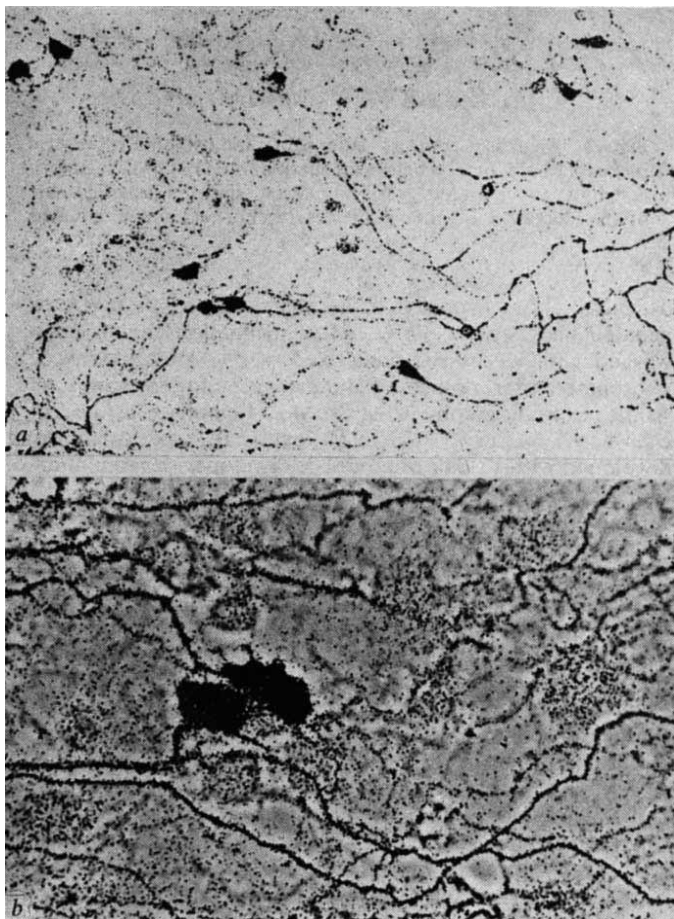


Fig. 1 Autoradiograph of myenteric plexus cultured for 10 d before incubation with ³H-GABA. *a*, Bright field photograph showing the border between the explant (left hand side) and the outgrowth zone (right hand side). Radioactivity is concentrated over 10 neuronal cell bodies, most of which lie within the explant, and processes which are mainly seen in the outgrowth zone. The light background labelling, which is most noticeable within the explant, is over non-neuronal cells ($\times 540$). *b*, Phase contrast photograph of two heavily labelled neurones and their processes lying in the outgrowth zone. Note other labelled processes as well as light labelling concentrated over the nuclear areas of non-neuronal cells which form a continuous sheet underlying the neuronal elements of the outgrowth zone. The camera was focused on the autoradiographic grains ($\times 720$).

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Fig. 2 A phase contrast autoradiograph of the myenteric plexus cultured for 12 d before incubation with ³H-GABA. Radioactivity is concentrated over one neurone and the proximal part of its process; the other neurones in the field are unlabelled (arrows) ($\times 630$).

described above except that the incubation solution was oxygenated and kept at 37 °C. After incubation the tissue was washed with Krebs solution, fixed in 4% glutaraldehyde in phosphate buffer, osmicated and processed for electron microscopic autoradiography using Ilford L-4 emulsion as described elsewhere⁹. After 21–28 days exposure, specimens were developed with Kodak Microdol-X developer. Examination of thin sections through individual ganglia of the plexus and the surrounding smooth muscle showed three degrees of labelling (Fig. 3): heavy labelling over approximately 5% of the neurones in the ganglia and over some axons in nerve bundles running through the smooth muscle coat, light labelling over the glial cells of the ganglia and almost total absence of label over the smooth muscle and fibroblasts outside the ganglia and most of the neurones within the ganglia. The labelled neurones were small to medium sized, as far as could be judged by the examination of single thin sections, and were often elongated and found at the surface of the ganglia as illustrated in Fig. 3. The cytoplasm was fairly dark, being packed with free ribosomes and rough endoplasmic reticulum and the mitochondria were small and electron dense. They most closely resembled neuronal type 1 in the ultrastructural classification of myenteric neurones proposed by Cook and G.B.¹⁰, the main discrepancy being the absence of accompanying satellite cells around the labelled neurones. The neuronal labelling, especially inside the ganglia, was greatly reduced if 10^{-3} M AChC was present in the incubation medium. Thus, there is good agreement between the results obtained from the *in situ* and *in vitro* experiments both with respect to the distribution of label and the effects of uptake inhibitors.

Current evidence indicates that the only vertebrate neurones to possess a high-affinity uptake mechanism for GABA are GABAergic neurones, although it is uncertain whether all GABAergic neurones share this property^{11,12}. This has recently received support from studies on ³H-GABA uptake into spinal cord neurones in culture, where it was shown that no identifiable cholinergic neurones showed high-affinity GABA uptake¹³. The results above therefore raised the question as to whether there

are GABAergic neurones in the myenteric plexus. If there are, the plexus should contain GAD and be able to accumulate newly synthesised GABA. Furthermore, it should contain endogenous GABA, and pharmacological actions of GABA in the gut ought to be detectable.

We measured the specific activity of GAD by formation of labelled GABA from labelled glutamic acid as described by others¹⁴, in three separate experiments. The plexus from the taenia coli of 1–8-day-old guinea pigs, dissected completely free of smooth muscle as described previously⁶, was homogenised in ice-cold distilled water and incubated at 37 °C for 15, 30 and 60 min in a solution containing the following components (final concentrations): potassium phosphate, pH 6.5, 0.1 M; dithiothreitol, 0.5 mM; pyridoxal phosphate, 0.5 mM; sodium L-glutamate, 25 mM; L-[G-³H]glutamic acid, 500 μ Ci ml⁻¹, electrophoretically purified (specific activity 31 Ci mmol⁻¹, Radiochemical Centre). The total amount of protein in each experiment was 30–50 μ g. This was divided into six samples and the levels of ³H-GABA obtained were 1.5 to 5 times higher than the assay blanks. Significant levels of GAD were detectable, the specific activity being 2.18 ± 1.2 μ mol per h per 0.1 g protein.

Neurones can not only synthesise but also accumulate in high concentrations the transmitter they use. We therefore investigated whether accumulation of GABA in intact cells of the myenteric plexus could be detected following incubation of the tissue with radiolabelled glutamic acid¹⁵. For comparison, we also studied slices of rat cerebellar cortex, which contains GABAergic neurones^{4,5}, and rat superior cervical ganglion,



Fig. 3 Electron microscopic autoradiograph of the myenteric plexus incubated *in situ* with ³H-GABA. The micrograph shows the edge of a ganglion with two slightly labelled glial cells (G), identifiable by the morphology of the nucleus³¹, a heavily labelled neurone, a fibroblast (F) in the interstitial space between the ganglion and the surrounding muscle, and a part of the muscle layer (M), all of which are virtually unlabelled ($\times 13,000$).

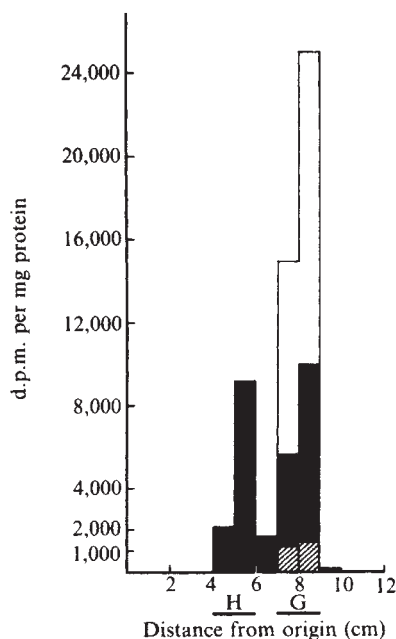


Fig. 4 Paper chromatography of ^3H -GABA and ^3H -homocarnosine synthesised from ^3H -glutamic acid in myenteric plexus (solid columns), cerebellum (open columns) and superior cervical ganglion (cross-hatched columns). The figure shows the distribution of radioactivity expressed as d.p.m. per mg protein based on results from three representative experiments, one for each tissue. The bars mark the position of non-radioactive homocarnosine (H) and GABA (G) on the chromatogram. The tissues were incubated in two changes of Eagle's minimum essential medium containing 6 g l^{-1} glucose and 10% fetal calf serum, for 40 min. The tissues were then placed in the same medium containing $4 \times 10^{-6}\text{ M}$ ^3H -glutamic acid, $100\text{ }\mu\text{Ci mmol}^{-1}$ (23 Ci mmol^{-1} ; Radiochemical Centre) for 17 h at 37°C . In one experiment electrophoretically purified ^3H -glutamic acid was used. After the incubation the samples were washed in two changes of phosphate-buffered saline, containing 150 mg l^{-1} non-radioactive glutamic acid, for 40 min and homogenised in $20\text{ }\mu\text{l}$ of ice-cold distilled water. A $5\text{-}\mu\text{l}$ sample of homogenate was withdrawn for protein estimation³² and $20\text{ }\mu\text{l}$ of 0.47 M formic acid, 1.4 M acetic acid containing 1 mg ml^{-1} of glutamic acid, GABA and in some cases homocarnosine was added to the remainder of the sample, which was frozen and thawed three times before being subjected to high-voltage paper electrophoresis³³. The paper was dried and ascending chromatography in *n*-butanol/acetic acid/water (60:15:25)¹⁷ was carried out at right angles to the direction of electrophoresis. The figure shows the distribution of radioactivity resulting from chromatography of that section of the paper to which non-radioactive GABA ran in the electrophoresis. In other experiments not shown here, *t*-butyl alcohol/methyl ethyl ketone/water/formic acid (8:6:5:1)²¹ was used as the solvent system. The amino compounds were visualised with ninhydrin spray, the paper cut into strips 1 cm long and 3.2 cm wide, the radioactivity eluted with 0.1 M HCl , followed by 4.5 ml of Aquasol (NEN) and counted in a scintillation counter.

where there is no evidence of GABAergic neurones^{16,17}. Isolated myenteric plexus, $400\text{-}\mu\text{m}$ thick slices of cerebellar cortex and desheathed superior cervical ganglia from 4–6-week-old rats were incubated with ^3H -glutamic acid for 17 h at 37°C . They were then homogenised and analysed for the presence of radioactive products of ^3H -glutamic acid by electrophoresis, chromatography in two different systems and subsequent scintillation counting. The results are shown in Fig. 4. The myenteric plexus, cerebellum and superior cervical ganglion all converted ^3H -glutamic acid to ^3H -GABA. The myenteric plexus contained $20,100 \pm 3,000\text{ d.p.m.}$ or $4.0 \times 10^{-13}\text{ mol}$ of ^3H -GABA per mg protein, the cerebellum contained $41,500 \pm 11,000\text{ d.p.m.}$ or $8.0 \times 10^{-13}\text{ mol}$ of ^3H -GABA per mg protein and the superior cervical ganglion contained $4,800 \pm 1,300$

d.p.m. or $9.6 \times 10^{-14}\text{ mol}$ per mg protein. Each value is an average of four experiments, all using the *n*-butanol/acetic acid/water chromatography system as described in Fig. 4 legend. In medium incubated without tissue no conversion was detectable. Thus, in these incubation conditions both the myenteric plexus and cerebellum contained significantly greater quantities of ^3H -GABA than the superior cervical ganglion, where synthesis and accumulation of ^3H -GABA apparently takes place in glial cells only^{16–19}.

In the case of myenteric plexus about 40% of the radioactivity running with non-radioactive GABA in the high-voltage electrophoresis separated from it in subsequent chromatography, forming a second peak of radioactivity. We found that homocarnosine (γ -aminobutyryl-L-histidine), a dipeptide which can be formed from GABA and histidine²⁰, also ran in the same position as GABA on the high-voltage electrophoresis and separated from it in two separate chromatography systems to run in an identical position to the second radioactive peak. We therefore conclude that this peak, which amounted to $13,400 \pm 2,200\text{ d.p.m.}$, is homocarnosine. The absence of ^3H -homocarnosine from the rat cerebellar cortex and superior cervical ganglion is not merely due to a species difference in GABA metabolism, as no ^3H -homocarnosine was detected in similar experiments with guinea pig superior cervical ganglia and cerebellar cortex, where similar levels of ^3H -GABA to those in the rat tissues were accumulated. We know of no other report on the synthesis or occurrence of homocarnosine in tissues other than brain. There it is present in low amounts but with heterogeneous distribution which, contrary to earlier beliefs, does not parallel that of GABA²¹. Homocarnosine is a potent anti-seizure agent^{22,23}, causes hyper-excitability and shortens barbiturate sleeping time²⁴ when injected into cerebral ventricles, and has been postulated to be a central neurotransmitter²⁵. Considerable evidence has also accumulated that carnosine, a related dipeptide (β -alanine-L-histidine), may be a neurotransmitter in the olfactory bulb²⁶.

When measuring endogenous GABA levels, the rapid and significant accumulation of GABA which takes place after the death of the animal, must be taken into account²⁷. Thus, GABA levels in the myenteric plexus would probably increase substantially during the time it takes to dissect it from the gut wall. To assess whether the plexus contained GABA we therefore compared GABA levels in strips of taenias containing the plexus to those in muscle (gastrocnemius) which does not contain the plexus, both tissues being removed from living anaesthetised guinea pigs and frozen immediately in liquid N_2 . The samples were then extracted twice with 80% ethanol, freeze dried and analysed for the presence of GABA with an automated amino acid analyser with a fluorimeter attached²⁷. Three experiments gave a value of $17.5 \pm 3.1\text{ nmol GABA per g wet weight of tissue}$ for taenia coli compared with $5.7 \pm 0.1\text{ nmol GABA per g wet weight of tissue}$ for muscle. The difference between the two tissues can probably be attributed to the myenteric plexus, in which case, if it were possible to measure endogenous GABA levels in the myenteric plexus alone, the values would be considerably higher than those reported here, as in the taenia the plexus is embedded in a large mass of muscle.

Few studies have been carried out on the action of GABA in the gastrointestinal tract. However, Hobbiger reported that 10^{-5} M GABA blocked the peristaltic reflex of the guinea pig ileum although it did not seem to act directly on smooth muscle. A variety of other effects of GABA on gut preparations have led to the suggestion that it may serve as a neurotransmitter^{28–30}.

Thus, we have shown that a small population of neurones in the myenteric plexus possesses a high-affinity uptake mechanism for GABA. These neurones are small to medium sized and share several common ultrastructural characteristics. Furthermore, the myenteric plexus contains GAD and endogenous GABA and can synthesise and accumulate ^3H -GABA and ^3H -homocarnosine from ^3H -glutamic acid. The complexities of GABA metabolism and compartmentation are such that these results should be interpreted with caution. However, in

conjunction with the pharmacological evidence that GABA affects intestinal peristalsis, the results suggest that it may have a role in neuronal transmission in the gut.

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Expression of a liver-specific function by mouse fibroblast nuclei transplanted into rat hepatoma cytoplasts

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Data from many somatic cell fusion experiments support the notion that the cytoplasm of eukaryotic cells contains elements which can alter gene expression and replication¹. More definitive evidence is perhaps lacking because the cell hybrids studied were complex cells comprising mixed genomes, frequently undergoing chromosomal losses and other aberrations, within mixed cytoplasts. Such problems have been alleviated by the development of the techniques of cybridisation, red cell-mediated microinjection and nuclear transplantation². Using this last technique, we showed that up to 40% of a monolayer culture of cytoplasts could be renucleated to form whole viable cells^{3,4}. Because of the high efficiency of fusion and the purity of cytoplast and karyoplast cultures used, large quantities of true cytoplasmic-nuclear hybrid cells suitable for immediate morphological and biochemical analysis could be formed. Here we describe the application of the technique to an investigation of the effects of rat liver cytoplasm on gene expression by mouse fibroblast nuclei. We show that the synthesis of mouse tryptophan amino transferase (TAT)—a liver-specific enzyme never detected in the fibroblast cell line was induced in hybrids constructed by transplantation of mouse (A9) fibroblast nuclei into rat liver (HTC) cytoplasts. Furthermore, as occurs in the rat cell line, its activity was modulated by addition of the synthetic corticosteroid, dexamethasone. Clearly, this hybrid cell system will be useful in the further characterisation and eventual isolation of the newly identified regulatory substance.

First, hybrid cells were formed and identified using our previously described techniques³. Briefly, cytoplasts were prepared from the HTC rat hepatoma cell line, which can be routinely enucleated with a >99% efficiency. Monolayer cultures of cytoplasts were treated with inactivated Sendai virus, after which excess unabsorbed virus was removed and replaced by a

suspension of karyoplasts prepared from the A9 mouse fibroblast cell line. After fusion, the dishes were washed vigorously to remove virus and excess unfused karyoplasts. Freshly prepared karyoplasts cannot adhere to a surface; only those fused to the cytoplast monolayer will remain associated with the dish. Approximately 20% of the HTC cytoplasts were routinely renucleated, with the value ranging from 10 to 30% in over 30 separate experiments. In cultures in which karyoplasts were layered over cytoplasts in the absence of virus, fewer than 1% of bodies contained nuclei, these cells being that small fraction of HTC cells not originally enucleated during centrifugation in medium containing cytochalasin B (see ref. 3 and below). Figure 1a shows an A9 nucleus-HTC cytoplast hybrid cell culture 18 h after fusion. By this time most cytoplasts not receiving nuclei have died and disintegrated. On the second day after fusion, medium containing 8-azaguanine was placed on the cells. The A9 nucleus does not code for the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT); thus, hybrids should also be resistant to the toxic analogue. As shown in Fig. 1b, the culture continued to grow (in fact, these cells were subcultured several times during the 2 weeks of growth), whereas a culture of whole HTC cells died within this time (Fig. 1c). The addition of azaguanine was delayed for 2 days to permit degradation of HGPRT contributed to the hybrids by the HTC cytoplasm³.

It was established, therefore, that the A9-HTC hybrids were viable and that they retained a genetic marker of the mouse nuclear donor cell. Furthermore, use of azaguanine permitted elimination of the small background of whole HTC cells originally contaminating the cytoplast cultures. As further proof that the cells contained mouse nuclei, karyotyping⁵ was carried out. Distinction between the two nuclei is possible as HTC cells contain several metacentric marker chromosomes⁶ whereas the mouse cells contain primarily telocentric chromosomes. Metaphase chromosome spreads were prepared from A9, HTC and hybrid cells (grown for 2 weeks) and scored for the presence of metacentric chromosomes. 100% of spreads from HTC cells contained metacentric chromosomes whereas 8% of spreads from A9 cells contained chromosomes that were scored as metacentric. Of all spreads examined from hybrid cells, only 4% contained chromosomes appearing metacentric. Thus, these results suggested that the constructed cells contained mouse, not rat, nuclei. Note also that hybrids could be formed, using similar techniques, by fusion of A9 cytoplasts and HTC karyoplasts. As expected, such hybrids were killed by 8-azaguanine.

With techniques for the routine preparation of hybrids now available, we assayed the cells for liver-specific functions. First,

Table 1 Histochemical assay for tyrosine amino transferase

Cells	Standard assay	Assay after heat treatment
Mouse fibroblast A9	—	—
Rat hepatoma HTC	++	—
A9 nucleus-HTC cytoplasm hybrids at		
24 h	+	+
48 h	+	+
72 h	+	+
96 h	+	+

Hybrids were constructed as described in Fig. 1. All cells (hybrids, A9s and HTCs) were treated for 24 h with 10^{-5} M dexamethasone before assay. The standard assay was a modification of the procedure described by Thompson and Tomkins⁷. Briefly, monolayers of cells were washed three times with buffer (1 M potassium phosphate, pH 7.6, 0.2 mM pyridoxal phosphate, 0.5 mM α -ketoglutarate) and allowed to air dry for 1 h at room temperature. The enzyme was then assayed as described⁷. For heat treatment, the monolayers were first heated to 75°C for 10 min and then assayed as above. The reaction product was visualised using a Zeiss research microscope. — Indicates that no reaction product could be seen in the cells; they appeared clear; ++ indicates an intense purple reaction product in the cells; + indicates a reaction which is less intense than the HTC cultures but still distinctly purple.

monolayer cultures were formed by transplantation of mouse fibroblast nuclei into rat hepatoma cytoplasts. At 0, 24, 48 and 72 h after fusion, a culture was incubated in dexamethasone for a further 24 h and then assayed histochemically⁷ for TAT activity. Cultures of whole A9 and HTC cells were similarly treated. As noted in Table 1, HTC cells exhibited a deep purple pigmentation but A9 cells were entirely clear. Hybrid cells, at all times measured, showed an intermediate level of staining. To determine whether the reaction product seen in the hybrids was due to the mouse liver form of the enzyme, and was not merely residual rat enzyme or a non-inducible mouse pseudoenzyme, heat inactivation experiments were carried out. Both the rat TAT and mouse pseudoenzymes are considerably more thermostable than the corresponding mouse liver enzyme. If the former enzymes are heated carefully to 75°C for 10 min, activity is markedly reduced; mouse liver TAT, however, retains much of its activity⁸. Therefore, monolayer cultures of hybrids, A9 and HTC cells were first heated to 75°C for 10 min and then assayed histochemically. As shown in Table 1, the appearance of reaction product in HTC cells was completely eliminated by heating. The intermediate level of response exhibited by hybrid cells was apparently unaffected. These results were obtained six times using hybrid cells constructed in separate experiments. In all the experiments presented in Table 1, cultures were treated with dexamethasone to maximise the intensity of the stain. However, in control cultures not treated with the steroid, A9 cells again exhibited no reaction product, HTC cells showed a distinctly purple but much less intense colour than induced cells, and the hybrids exhibited a barely detectable stain. It therefore seemed that the mouse enzyme produced was not only heat stable but also inducible. The magnitude of the induction was quantitated in later cultures as described below.

The observations were confirmed and quantitated using an *in vitro* biochemical assay for enzyme activity^{9,10}. In these experiments, carried out on 10 separate occasions, cells were assayed at various times during the second and sixth week after fusion. As summarised in Table 2, the hybrids formed by transplantation of A9 nuclei into HTC cytoplasts exhibited levels of enzyme activity greater than 25% of that shown by induced HTC cells. Note that (1) the hybrids could be grown in the presence of azaguanine, and that (2) TAT-producing cultures were confirmed as mouse cells by karyotyping, as described above. Furthermore, the indication that the enzyme made was indeed the steroid-inducible liver form of the enzyme was confirmed by experiments showing that the enzyme produced by

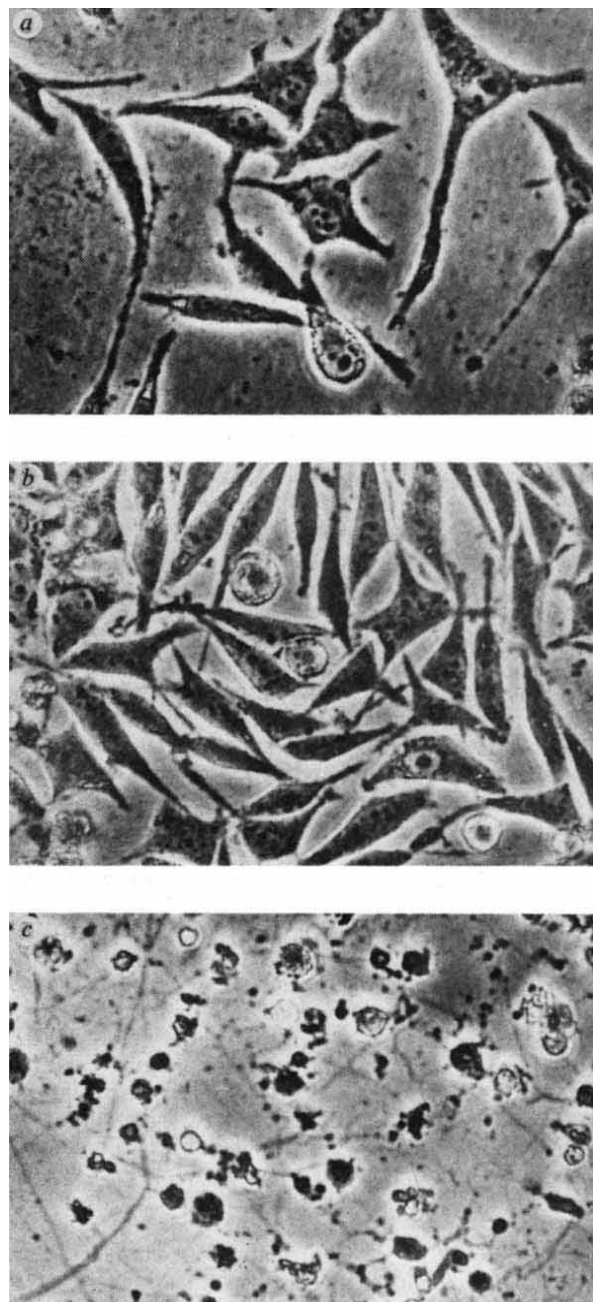


Fig. 1 Hybrid cells constructed by transplantation of mouse fibroblast A9 nuclei into rat hepatoma HTC cytoplasts. *a*, A culture at 18 h after formation; *b*, the same culture after 2 weeks of growth in azaguanine (after several subcultivations); *c*, HTC cells after 1 week in azaguanine (without subcultivation). Magnification, $\times 150$. The A9 parent cell line was maintained in monolayers in Dulbecco's modified Eagle's medium supplemented with 10% calf serum; the HTC parent line was grown in Waymouth's MAB 87/3 supplemented with 10% fetal calf serum. Previously described techniques were adapted for cytochalasin B-induced enucleation of the A9 and HTC cell lines. Cytoplasts and karyoplasts were then fused by Sendai virus-mediated fusion as described elsewhere^{3,12}.

hybrid cells was precipitable by antiserum against purified rat liver TAT. As shown previously⁸, such antiserum effectively precipitates both rat and mouse liver TATs, but not non-inducible mouse pseudoenzyme.

All the results in Table 2 were obtained from cultures between the second and sixth weeks after fusion. At earlier times it was difficult to obtain the number of hybrids needed for this biochemical assay. However, we considered it essential to assay

Table 2 Enzymatic assay of tyrosine amino transferase

Cells	Milliunits enzyme activity per 5×10^6 cells	
	-Dexamethasone	+Dexamethasone
Mouse fibroblast A9*	1.30	1.24
Rat hepatoma HTC*	6.80	38.69
A9 nucleus-HTC cytoplasm hybrids†	3.77	10.65
HTC nucleus-A9 cytoplasm hybrids‡	Not done	45.08

Hybrids were constructed as described in Fig. 1. Assays were carried out with and without a 24-h treatment with dexamethasone (10^{-5} M). Cytoplasmic extracts prepared from cells were assayed for TAT by a modification of the Diamondstone assay⁹, as described by Granner and Tomkins¹⁰. Assays were carried out on hybrids at various times during the second and sixth weeks after fusion.

* Data represent means of 10 separate assays.

† Data represent means obtained from 10 separate fusion experiments.

‡ Datum represents mean obtained from three separate fusion experiments.

early hybrids by the more accurate, quantitative *in vitro* assay. Therefore, 15 separate cultures of HTC cytoplasts were fused to A9 karyoplasts and pooled at 24 h after preparation. (Because of the ratio of karyoplasts to cytoplasts required to obtain maximum fusion, it was necessary to prepare A9 karyoplasts by enucleation of 128 cultures.) The steroid-induced level of activity in HTC cells was 28.4 mU per 5×10^6 cells, whereas the activity in 24-h hybrid cells was 7.6 mU per 5×10^6 cells. Three-week-old hybrids assayed at the same time yielded 6.4 mU of activity. Thus, the levels of enzyme produced were apparently similar throughout the 6 weeks of study. Furthermore, the enzyme present in these early hybrids was shown to be the heat-stable mouse liver form of the enzyme: after heat treatment which reduced the rat HTC enzyme activity to background levels (<1 mU per 5×10^6 cells), the hybrid cell enzyme retained, as predicted, 30% of its activity (see ref. 8). Also noted in Table 2 are observations that hybrids constructed by transplantation of HTC nuclei into A9 cytoplasts produced enzyme at levels comparable to those of the parent rat liver cell.

The data presented here were obtained from cells assayed up to 6 weeks after fusion. In all 10 experiments in which A9 nuclei were transplanted into HTC cytoplasts, however, the hybrids abruptly ceased making TAT between 6 and 8 weeks after fusion. The loss of activity was correlated with a transient change in morphology and death of many cells, after which the cultures continued to grow, apparently indefinitely, without detectable TAT activity. The period during which TAT was expressed represented some 75–100 cell generations. This would be sufficient to result in a greater than 10^{20} -fold dilution of cytoplasmic elements originally present in the hepatoma cytoplasm donor cell.

This system, exhibiting the induction and modulation of an activity not normally expressed by the nuclear parent, is ideal for further analysis of the cytoplasmic elements capable of altering gene expression. Moreover, the induced programme of expression is apparently specific. Thus, the hybrids did not synthesise albumin, a liver-specific function not retained by HTC cells grown in culture (data not shown). We are now attempting to induce the synthesis of TAT in A9 cells by microinjection of components prepared from the cytoplasm of liver cells, using techniques we previously used to microinject cytoplasmic elements capable of inducing DNA synthesis in dormant nuclei¹¹. If successful, such experiments would provide the basis for an assay system useful for the purification of the inducing substance.

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Isolation by sucrose-density fractionation and cultivation *in vitro* of actinomycetes from nitrogen-fixing root nodules

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The fixation of atmospheric nitrogen by actinomycete-nodulated, woody dicotyledonous plants represents a substantial contribution to the global nitrogen cycle¹, contributing especially to forested areas, wetlands, fields and disturbed sites of temperate and tropical regions². The study of these nitrogen-fixing symbioses has been hampered by difficulties in isolating and culturing the microsymbionts *in vitro*. Recently, root nodule actinomycetes have been isolated by microdissection techniques and cultured *in vitro*^{3,4}. We report here the isolation and culture for the first time of the actinomycetes associated with root nodules of *Elaeagnus umbellata* (Elaeagnaceae) and *Alnus viridis* ssp. *crispa* (Betulaceae) (shown in Fig. 1a and b, respectively), using a technique radically different from those used previously. For separating actinomycetes from root nodules, we have used the simple fractionation technique of sucrose-density sedimentation.

Root nodules (0.25 g fresh weight) from plants of *E. umbellata* Thunb., growing in nitrogen-free one-quarter-strength Hoagland's water culture solution⁵, were surface-sterilised for 15 min in 50 ml of a 30% (v/v) solution of Clorox (5.25% NaOCl) to which one drop of Tween 20 had been added as a surfactant. The nodules were washed twice in sterile distilled water and then crushed in a sterile mortar and pestle with a small amount of sterile distilled water. The resultant slurry was filtered serially through Miracloth (Chicopee Mills) and glass wool and diluted to 10 ml with sterile distilled water.

Cellulose-nitrate centrifuge tubes were sterilised with 95% ethanol and permitted to air dry. Using heat-sterilised sucrose solutions, discontinuous density gradients were constructed of a 3-ml cushion of 60% (w/v) sucrose, a 3-ml layer of 45% (w/v) sucrose and a 6-ml layer of 30% (w/v) sucrose. Two ml of the filtered nodule suspension were layered onto each gradient, and the gradients centrifuged at 100,000g for 3 h at 4°C in a Beckman L2-65B ultracentrifuge equipped with a SW-40 rotor. The gradients were fractionated by aseptically piercing the bottoms of the tubes with a 25-gauge needle and collecting eight

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1-ml fractions. The sucrose-density fractions were used to inoculate agar plates of nutrient medium containing yeast extract 0.5%, dextrose 1.0%, casamino acids 0.5%, H_3BO_3 1.5 mg l^{-1} , $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 1.5 mg l^{-1} , $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$ 4.5 mg l^{-1} , $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ 0.25 mg l^{-1} , $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.04 mg l^{-1} , vitamin B_{12} 1.6 mg l^{-1} and Difco agar 0.8%. The plates were sealed with Parafilm and incubated at 28°C .

Colonies of a finely filamentous actinomycete appeared on the inoculation plates after approximately 3–5 weeks. The frequency of this organism in the sucrose-density fractions as demonstrated by its growth on the isolation plates was greatest in fraction 4, corresponding to the interface between the 60% and 45% sucrose layers. At 8 weeks this '*Elaeagnus* isolate' was subcultured to agar and liquid media for further observation and characterisation. The isolate grows in dense colonies composed of branching septate filaments which have diameters of approximately $0.3 \mu\text{m}$ (Fig. 1a). Sporangia, attached by a thickened sporangiophore, are produced terminally on the submerged filaments. Spores within the sporangia are roughly $1 \mu\text{m}$ in diameter and spherical to oblong. The *Elaeagnus* isolate will grow on the surface of agar, but true aerial mycelia or aerial

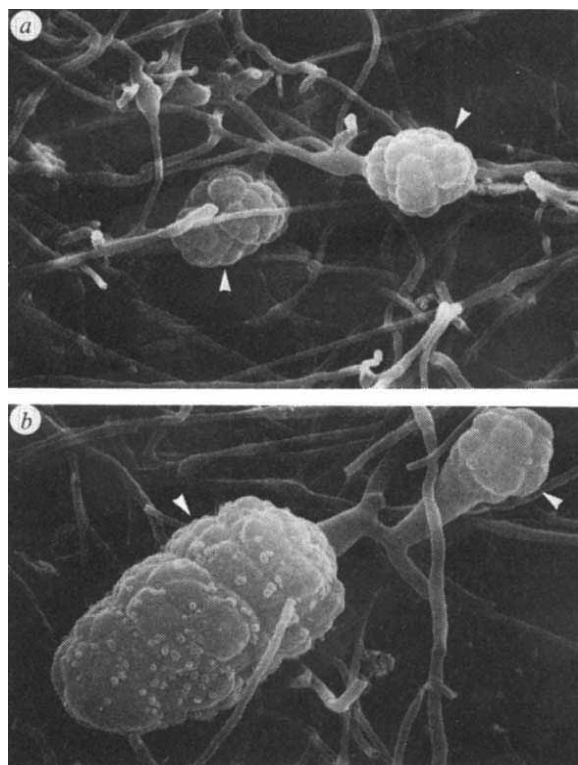


Fig. 1 Scanning electron micrographs of *in vitro* cultured actinomycete microsymbionts, $\times 3,550$ (specimens fixed in glutaraldehyde, dehydrated in ethanol, critical-point dried with liquid CO_2 and sputter-coated with gold-palladium). *a*, Actinomycete isolated from root nodules of *E. umbellata*. Sporangia (arrows) contain many small spores. *b*, Actinomycete isolated from root nodules of *A. viridis* ssp. *crispa*. Arrows indicate sporangia.

sporangia are not formed. Colonies produce variable amounts of blood-red pigment that diffuses into the culture medium. We have designated the isolate *Frankia* sp. EuII until the uncertainty of specific epithets⁶ can be resolved.

Infectivity tests were carried out to confirm that the *Elaeagnus* isolate was in fact a symbiotic endophyte. *Elaeagnus* seedlings, germinated and grown in moist sand for 4 weeks, were transplanted into 400 ml standing water cultures supplied with Hoagland's nitrogen-free solution. The plants were grown in a controlled-environment chamber under a light-dark cycle for 16 h and 8 h, respectively, and a day-night temperature cycle of 27°C and 21°C , respectively. After 1 week in the nitrogen-free

solution, the plants were inoculated with a small amount of washed, homogenised *Elaeagnus* isolate from 4–6-week old broth cultures. Control plants were either uninoculated or inoculated with a crushed nodule inoculum (0.3 g ground *Elaeagnus* nodules in 5 ml water).

After 4 weeks, young developing nodules were evident on many inoculated plants. Uninoculated control plants bore no nodules. At 8 weeks, plants inoculated with a crushed-nodule inoculum were beginning to recover from nitrogen starvation whereas those inoculated with the *Elaeagnus* isolate showed no signs of recovery. At this time the number of nodules per plant was counted and routine acetylene reduction tests⁷ were carried out on the observed nodules to determine nitrogenase activity (Table 1). Nodules initiated by the crushed nodule inoculum exhibited acetylene reduction rates comparable to previous reduction values for other actinomycete-nodulated plant species⁸. Nodules initiated by the *Elaeagnus* isolate showed no measurable acetylene-reduction activity and were clearly ineffective.

Figure 2a shows the endophyte within host cells of a plant inoculated with a crushed nodule suspension. Figure 2b shows the endophyte within nodule cells of a plant inoculated with *Frankia* sp. EuII. Typical vesicles seen in the nodules initiated by a crushed nodule inoculum (Fig. 2a) were absent in the nodules initiated by the *Elaeagnus* isolate (Fig. 2b).

In view of the above successful isolation, described sucrose-density fractionation procedure was repeated step-by-step using 0.5 g (fresh weight) of nodules from *Alnus viridis* ssp. *crispa* (Aiton) Turill collected near Atikokan, Ontario, Canada. After 3 weeks of incubation, a finely filamentous actinomycete was observed growing on the agar plates inoculated with sucrose-gradient fraction 4.

Like the *Elaeagnus* isolate, the '*Alnus* isolate' grows in radial colonies composed of branching septate filaments bearing sporangia, attached by a thickened sporangiophore (Fig. 1b). However, unlike species EuII, the *Alnus* isolate grows more diffusely, will not grow on the surface of agar and does not produce any pigments. Filament diameter of the *Alnus* isolate is approximately $1 \mu\text{m}$ but sporangia are much larger ($20\text{--}60 \mu\text{m}$, Fig. 1b) than those of the previously isolated nodule actinomycetes. Spores are slightly smaller than $1 \mu\text{m}$. We have designated this isolate *Frankia* sp. AvcII.

Infectivity tests were carried out using the *Alnus* isolate or a crushed nodule suspension (0.7 g ground *Alnus* nodules in 5 ml distilled water). After 10 d young developing nodules were evident on inoculated plants. Uninoculated control plants bore no nodules. At 4 weeks the number of nodules per plant was counted and acetylene reduction assays were carried out on the observed nodules (Table 1). Acetylene-reduction values of the isolate-inoculated plants were greater than those of plants inoculated with nodule suspensions. When endophyte morphology was observed by scanning electron microscopy, the

Table 1 Nodulation and acetylene reduction values for seedlings of *Elaeagnus* and *Alnus*

Treatment	No. of test plants	Plants nodulated (%)	Nodules per plant*	Acetylene reduction†
<i>Elaeagnus</i> , uninoculated	6	0	0	—
<i>Elaeagnus</i> , nodule suspension	15	87	9.9	4.7–5.0
<i>Elaeagnus</i> , <i>Frankia</i> sp. EuII	30	33	6.2	0
<i>Alnus</i> , uninoculated	6	0	0	—
<i>Alnus</i> , nodule suspension	15	20	1.3	Negligible
<i>Alnus</i> , <i>Frankia</i> sp. AvcII	30	100	22.7	0.46–2.68

* Values are expressed as an average computed from those plants bearing root nodules and do not include unnodulated plants.

† Values are expressed as μM ethylene per g freshweight per h; uninoculated control plants were not measured for reductin activities.

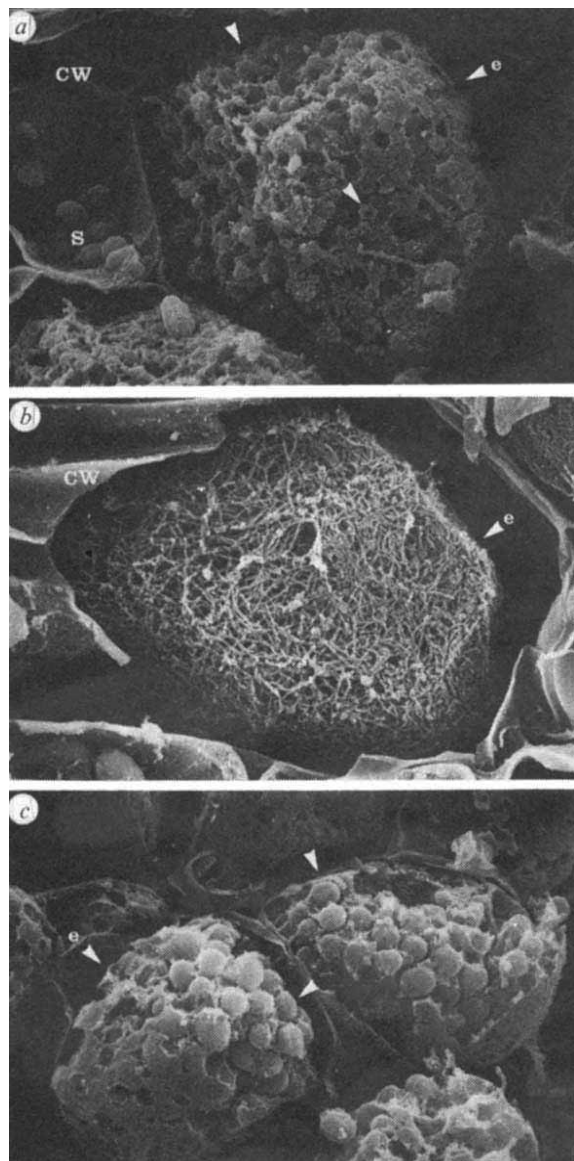


Fig. 2 Scanning electron micrographs of infected cells of host plant root nodules, $\times 1,100$ (samples prepared as in Fig. 1); e, endophyte cluster; cw, host cell wall; s, starch grain. a, Actinomycete endophyte within cells of *Elaeagnus* inoculated with a crushed nodule suspension. Vesicles (small arrows) are borne terminally on mycelial branches. b, Actinomycete endophyte within cells of *Elaeagnus* inoculated with *Frankia* sp. EuII. Note lack of endophytic vesicles. c, Actinomycete within cells of *Alnus* inoculated with *Frankia* sp. AvcII. Vesicles (small arrows) are shown embedded in coagulated host cell cytoplasm.

nodules initiated by the *Alnus* isolate contained the vesicular endophyte typical of the *Alnus* symbiosis (Fig. 2c). These results suggest that the ineffective nature of the *Elaeagnus* isolate is peculiar to that organism and is not a result of the isolation procedure.

The technique described here is a relatively simple procedure for the isolation of actinomycete endophytes. The use of sucrose-density fractionation has also been applied to rhizobial-legume symbioses^{9,10} and we are attempting isolations from other actinomycete-nodulated plants using this technique. Density sedimentation has proved superior to a previously used fractionation technique¹¹ for endophyte isolation.

The actinomycetes that we have isolated and cultured *in vitro* show a striking morphological similarity to the *Elaeagnus* and *Alnus* endophytes found in nodules of field-collected plants and each is able to reinfect the corresponding seedlings grown in water culture.

The fact that the *Elaeagnus* isolate does not form vesicles and does not reduce dinitrogen is particularly interesting in view of the postulated role of the vesicles in nitrogenase activity. Vesicles have been implicated as the site of nitrogen fixation¹² and previous research has shown that ineffective, non-vesiculate strains may exist¹³. The fact that this infective actinomycete fails to form vesicles and is incapable of fixing atmospheric nitrogen strengthens the hypothesis that the vesicle is the site of the nitrogenase enzyme.

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Dual origin of recent Dutch elm disease outbreaks in Europe

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Dutch elm disease was first recorded in 1918 in Western Europe and quickly spread to the US. The origin of the causal fungus, *Ceratocystis ulmi*, is unknown. A serious new epidemic began in the UK around 1970 and led to the discovery that there were two strains of the fungus: a highly pathogenic 'aggressive strain' responsible for the epidemic and thought to have been imported from North America^{1,2}, and a less pathogenic 'non-aggressive strain' believed to be endemic to the UK and adjacent parts of Europe since the 1920s (refs 1, 2). Recent disease outbreaks associated with the appearance of the aggressive strain in Holland³ (1972), France^{4,5} and Germany^{6,7} (1973) were assumed to be secondary foci of the UK epidemic. A similar outbreak in Italy⁷ (1973), however, seemed to be of longer standing (L. Mittemberger and O. Rackham, personal communications) and this, together with the discovery of the aggressive strain in Iran in 1975 (ref. 8), suggested that there was more than one source of the aggressive strain. I show here that there are two distinct races of the aggressive strain and that they have probably entered Europe independently.

When the colony morphologies of aggressive isolates were examined, those from the US, Canada, Holland, France and Germany were found to resemble closely UK isolates, being regular and striate, whereas those from Italy were uneven and less striate. Moreover, isolates obtained from Iran in 1975 were of the same morphological type as the Italian isolates, and a survey in 1977 confirmed the widespread occurrence of the Italian type in Iran⁸.

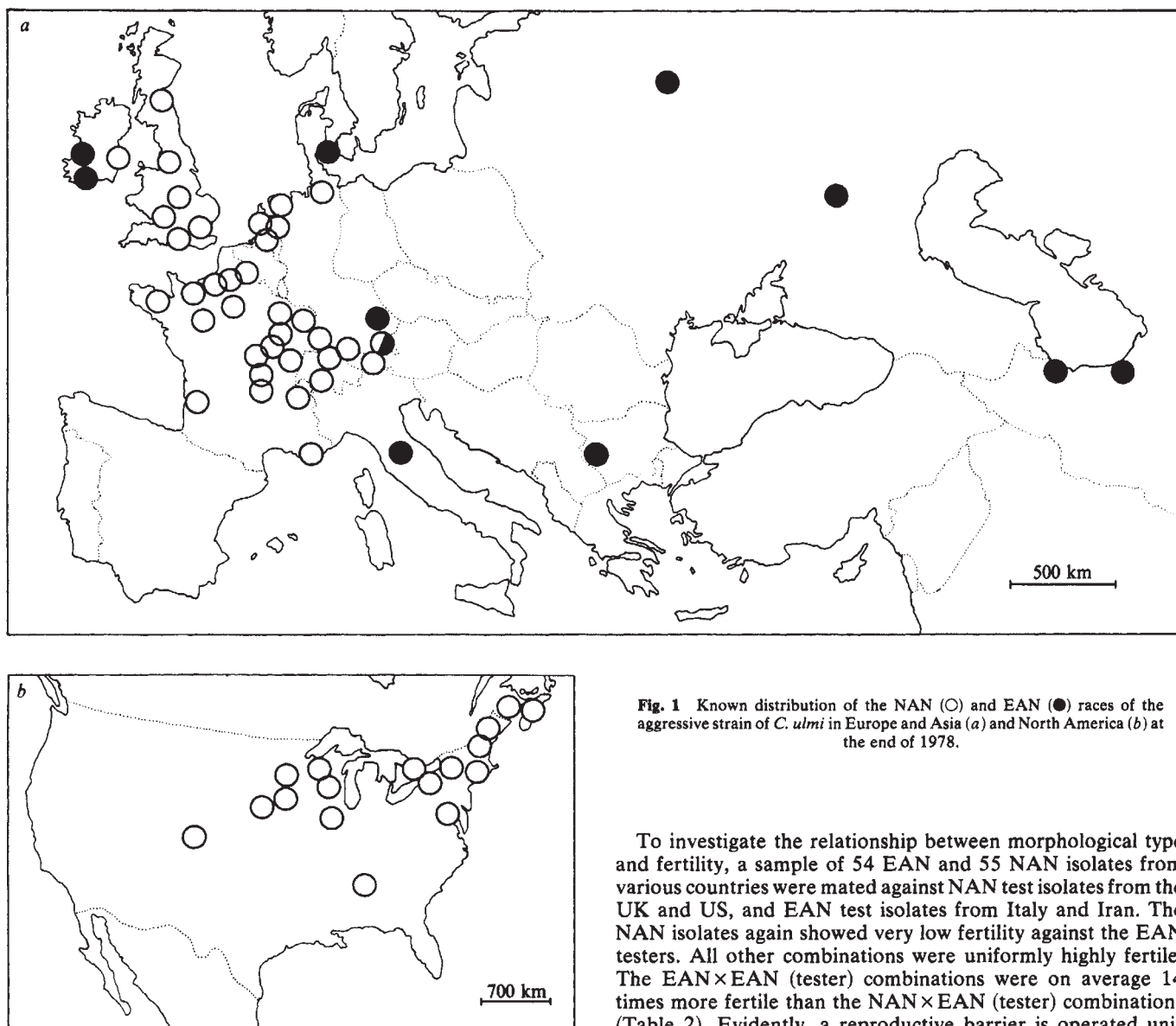


Fig. 1 Known distribution of the NAN (○) and EAN (●) races of the aggressive strain of *C. ulmi* in Europe and Asia (a) and North America (b) at the end of 1978.

Striking differences between the two morphological types were found when reciprocal fertilisations were carried out between aggressive isolates from the UK, US, Italy and Iran. *C. ulmi* has two mating types, termed A and B. Using one isolate of each mating type from each country, donor spores of one mating type were applied to fertilise a defined area on the surface of a recipient culture of the other mating type⁹. The mean number of perithecia (the sexual stage)¹⁰ resulting per cm² was used as an index of the relative fertility of a given combination. The UK and US isolates were highly fertile with all donor isolates, whereas Italian and Iranian isolates showed low fertility with the UK and US isolates and high fertility with each other, the latter combinations being on average 18 times more fertile (Table 1). This indicated that the two morphological types were separate genetic entities, and they were provisionally termed the North American (NAN) and Eurasian (EAN) races of the aggressive strain.

Further samples of the aggressive strain were obtained from sites throughout North America and Europe. They included isolates from new outbreaks associated with the appearance of the aggressive strain in Ireland¹¹ (1977) and Denmark (1978) and isolates from the USSR and Bulgaria. Of 201 isolates examined morphologically, 127 were classified as NAN and 74 as EAN (Table 2).

To investigate the relationship between morphological type and fertility, a sample of 54 EAN and 55 NAN isolates from various countries were mated against NAN test isolates from the UK and US, and EAN test isolates from Italy and Iran. The NAN isolates again showed very low fertility against the EAN testers. All other combinations were uniformly highly fertile. The EAN × EAN (tester) combinations were on average 14 times more fertile than the NAN × EAN (tester) combinations (Table 2). Evidently, a reproductive barrier is operated unidirectionally by the EAN against the NAN isolates, a classic form of genetic isolation. The clear correlation between morphological type and relative fertility confirmed the NAN and EAN as separate genetic entities.

The NAN is the morphological type originally described in 1973 (ref. 1). The EAN differs from the NAN in having a less distinctive striate-petalloid pattern, the colonies often being lobed and uneven. In growth rate tests at 20 °C EAN isolates grow marginally more slowly than NAN isolates. Fresh EAN isolates often sector to a 'uniform powdery' (*up*) mutant, thought to be cytoplasmic in origin and the cause of their unevenness and slower growth.

That the NAN and EAN should be considered separate races within the aggressive strain rather than separate strains of *C. ulmi* is demonstrated by their similarities. In a preliminary pathogenicity test both types were highly pathogenic to *Ulmus procera*. In each, the B-mating type predominates and the rarer A-type characteristically produces protoperithecia on malt agar⁸⁻¹⁰. Both exhibit the unusual phenomenon of pseudoselfing¹⁰; both show extensive genetic isolation from the non-aggressive strain (refs 9 and 12 and C.M.B., unpublished); both have an optimum temperature for growth of ~20–22 °C compared with ~30 °C for the non-aggressive strain (J. Lea and C.M.B., unpublished).

As Fig. 1 shows, US, Canadian, UK, French, Dutch, Austrian and Swiss isolates are exclusively of the NAN race whereas Italian, Danish, Bulgarian, USSR and Iranian isolates are

Table 1 Frequency of perithecia (mean per cm²) in inter-fertility tests of aggressive isolates of *C. ulmi* from the UK, US, Italy and Iran

		B-type donor isolates				<i>t</i> -tests for differences between means of UK and US against Italy and Iran
		UK	US	Italy	Iran	
A-type recipient isolates	UK	451	387	381	423	NS
	US	189	186	228	173	NS
	Italy	3	0	56	41	†
	Iran	75	29	246	305	*
		A-type donor isolates				
		UK	US	Italy	Iran	
B-type recipient isolates	UK	198	281	239	284	NS
	US	43	24	30	32	NS
	Italy	115	55	357	368	*
	Iran	32	5	309	251	†

The experiment was carried out on elm sapwood agar. Each figure is the mean of four replicates. Student *t*-tests were carried out on replicate data transformed to square roots; significance levels: NS, not significant; * $P < 0.01$; † $P < 0.001$.

exclusively EAN. With the exception of Ireland, therefore, NAN shows a distinctly westerly and EAN a distinctly easterly distribution, both races occurring at Rosenheim in south-east Germany (5 EAN and 5 NAN in a sample of 10 isolates in August 1978).

On this evidence I suggest that the recent outbreaks of Dutch elm disease in Western Europe are of dual origin: through the importation of the NAN race of the aggressive strain from North America into the UK in the late 1960s (ref. 2), and into France, Holland and Germany, probably via Britain, in the early 1970s; and through migration of the EAN race from Italy, from central Europe or from farther east.

The Irish outbreak of 1977 is particularly interesting. The NAN isolates came from the Dublin area, whereas the EAN isolates all came from the Limerick-Cork area in the south-west. The Dublin and Limerick outbreaks were thought by Walsh and Mangan¹¹ to have originated independently, and the geographical distribution of the two races seems to confirm this. It would be intriguing to know the origins of these outbreaks. The Danish outbreak of 1978 is so far confined to Odense on the

island of Funen, and because it is caused by the EAN race is probably due to importation of diseased material from the south or east. These events indicate that had the aggressive strain not reached the UK in the form of the NAN race from North America, it would probably have eventually arrived in the form of the EAN race from south or central Europe.

Further surveys of the fungus are now required in Europe and Asia, in particular Siberia, the Himalayas and China. These are the main sources of resistant elm material and somewhere in this region may be the centre of origin of *C. ulmi*. The full extent of variation in the fungus needs to be determined as soon as possible for the purposes of breeding resistant elms. Promising elm material should now be screened with both races of the aggressive strain.

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Energy dependence and reversibility of membrane alterations induced by polyene macrolide antibiotics in *Chlorella vulgaris*

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Table 2 Separation of aggressive isolates of *C. ulmi* into the NAN and EAN morphological types, and results of fertility tests against a recipient EAN isolate

Country	Morphological type			Fertility tests against EAN isolate* Mean perithecia per cm ²	
	No. of isolates examined	No. of NAN	No. of EAN	NAN isolates (no. of isolates tested)	EAN isolates
Austria	2	2	—	3.5(2)	—
Bulgaria	5	—	5	—	95.0(5)
Canada	7	7	—	3.5(2)	—
Denmark	3	—	3	—	70.4(3)
France	45	45	—	2.7(15)	—
Germany	22	15	7	2.1(6)	41.2(7)
Holland	13	13	—	5.5(8)	—
Ireland	27	7	20	5.9(7)	49.3(20)
Iran	32	—	32	—	35.6(13)
Italy	4	—	4	—	43.7(3)
USSR	3	—	3	—	58.7(3)
Switzerland	2	2	—	5.0(3)	—
US	36	36	—	2.1(6)	—
UK	(>4,000)	(>4,000)	—	4.0(8)	—
				Mean 3.6(55)	50.6(54)

* Isolates were from twig samples from individual diseased trees with the exception of two from the USSR which were bark isolates. Over 4,000 twig and bark isolates have been examined in the UK since 1972.

† The recipient EAN isolate used as a standard was Iranian isolate AST 20 ('A' mating type). All isolates tested against it were 'B' mating type. The data are from five separate experiments carried out on elm sapwood agar.

The requirement of metabolic energy for the interaction of polyene macrolide antibiotics with eukaryotic organisms remains a controversial subject (for review see ref. 1). It has been claimed that the lethal binding of these antibiotics to the sterol target component of the hydrophobic core of the membrane, in accordance with the model of de Kruijff and Demel², is an energy-dependent process. When energy production is reduced by removal of all metabolisable substrates or by adding metabolic inhibitors, polyene binding and antifungal effects are also reduced. Metabolic energy may be required to maintain binding site accessibility or to move antibiotic molecules to the active site. The interaction is also restricted at low temperatures, possibly because of the reduced thermal mobilities of the groups concerned with antibiotic uptake. However, it should be emphasised that the interaction of polyene macrolides with artificial lipid membranes is a purely physicochemical process, although the type of permeability pathways induced are similar to those observed in natural membranes¹. Using *Chlorella vulgaris* as a model organism, we demonstrate here that the interaction of polyene macrolides with sensitive cells and the induction of lethal membrane permeability changes are energy-dependent processes or purely physicochemical phenomena, depending on the structure of the antibiotic used.

As *C. vulgaris* is an autotrophic aerobic organism, energy production can be controlled naturally by changing the availability of light and oxygen, thus eliminating the need to use

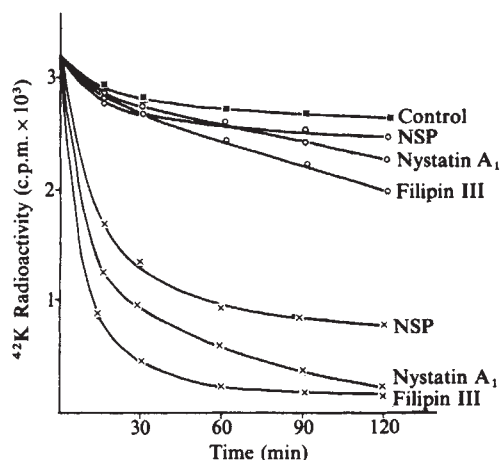


Fig. 1 The influence of temperature on the leakage of potassium from *C. vulgaris* on treatment with filipin III ($30 \mu\text{g ml}^{-1}$), nystatin A_1 ($10 \mu\text{g ml}^{-1}$) and *N*-succinylperimycin (NSP) ($25 \mu\text{g ml}^{-1}$). ^{42}K -labelled *C. vulgaris* cells (3 mg ml^{-1}) were suspended in Tris-HCl buffer, pH 7.2, at 0°C ($\circ$) or 30°C ($\times$), and 0.1-ml samples of antibiotic solution in dimethyl sulphoxide (DMSO) added. The suspensions were aerated by shaking and illuminated. Aliquots were taken, cells filtered and washed with ice water and their radioactivity recorded.

chemical inhibitors which are not usually very specific. In anaerobic conditions and in the absence of light, ATP production in *Chlorella* spp. is completely inhibited⁵. The presence of ergosterol in the cytoplasmic membrane⁴ makes this organism sensitive to the action of polyene macrolides^{5,6}.

In this study we have used compounds which represent possible different types of interaction with *C. vulgaris*, based on the reversibility of the lethal action of polyene macrolides on this organism. Following our observation on the reversibility of fungicidal action of a polyene macrolide⁷, we have demonstrated that the ability of *C. vulgaris* cells to repair antibiotic-induced damage⁶ depends on the structure of these antibiotics. Small macrolide ring polyenes (for example, filipin III) act irreversibly, whereas the action of those with a large macrolide ring but with low unsaturation in the chromophore (for example, nystatin A_1) can be reversed after brief contact with cells but not

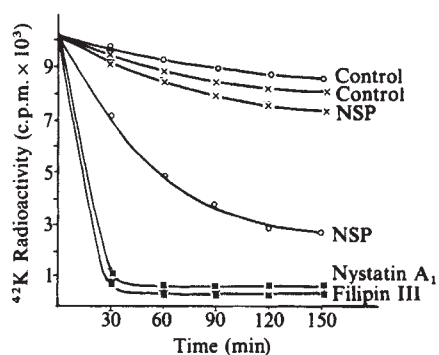


Fig. 2 The influence of the metabolic inhibitor, CCCP, on the potassium leakage from *C. vulgaris* treated with filipin III ($50 \mu\text{g ml}^{-1}$), nystatin A_1 ($20 \mu\text{g ml}^{-1}$) and *N*-succinylperimycin (NSP) ($25 \mu\text{g ml}^{-1}$). ^{42}K -labelled *C. vulgaris* cells (3 mg ml^{-1}) were suspended in Tris-HCl buffer pH 7.2, at 30°C and preincubated with ($\times$) and without ($\circ$) $5 \times 10^{-3} \text{ M}$ CCCP for 30 min with shaking and illumination, followed by the addition of antibiotics in 0.1 ml DMSO. Aliquots were taken, cells filtered and washed with ice water and their radioactivity recorded.

after prolonged exposure. Compounds with a large macrolide ring and a heptaenic chromophore (for example, *N*-succinylperimycin) induce repairable membrane alterations, and their lethal action can be fully reversed after removal of the antibiotic from the medium, regardless of the time of exposure.

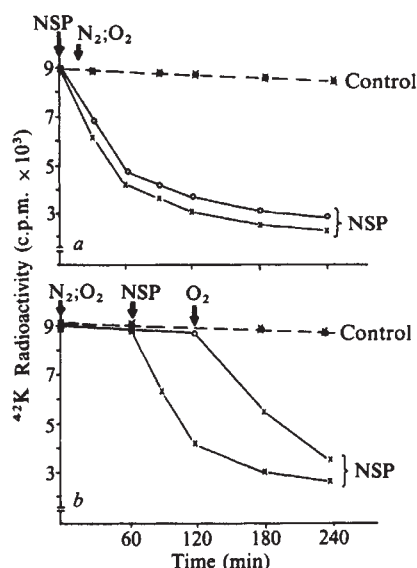


Fig. 3 The influence of light and oxygen on potassium leakage from *C. vulgaris* on treatment with *N*-succinylperimycin (NSP) ($25 \mu\text{g ml}^{-1}$). ^{42}K -labelled *C. vulgaris* cells (3 mg ml^{-1}) were suspended in Tris-HCl buffer, pH 7.2, at 30°C . *a*, The suspension was aerated by shaking and illuminated, then a 0.1-ml solution of NSP in DMSO added. After 15 min, nitrogen was passed through the suspension in the dark ($\circ$) or the culture was kept in the light and aerated ($\times$). Aliquots were taken, the cells filtered, washed with ice water and dried, and their radioactivity recorded. *b*, The suspension was kept in nitrogen in the dark, with shaking, or aerated under illumination. After 1 h NSP, $25 \mu\text{g ml}^{-1}$, in 0.1 ml DMSO was added to both suspensions. After another hour, air was introduced to the anaerobic suspension and the suspension was shaken and illuminated. Aliquots were taken from both suspensions, the cells filtered, washed with ice water and dried, and their radioactivity recorded. $\circ$, N_2 ; $\times$, O_2 .

The interaction between the compounds examined and *C. vulgaris* was followed by measuring the leakage of potassium ions. The discharge of the gradient of this marker ion may be regarded as one of the first indications of membrane damage⁸. Filipin III⁹, nystatin A_1 (ref. 10) and *N*-succinylperimycin^{11,12} induce potassium leakage only at elevated temperatures (Fig. 1). In these conditions the use of the metabolic inhibitor carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) does not prevent the interaction of filipin III and nystatin A_1 with *C. vulgaris*, but strongly inhibits the activity of *N*-succinylperimycin (Fig. 2). The leakage of potassium from cells treated with filipin III and nystatin A_1 , in the presence of CCCP, indicates that the interaction of these antibiotics with the plasma membrane of *C. vulgaris* is a physicochemical phenomenon, not an energy-dependent process. The energy dependence of the interaction of *N*-succinylperimycin with the membrane was further confirmed by changing the availability of light and oxygen (Fig. 3). Whether the cells were treated with *N*-succinylperimycin in aerobic conditions and after 20 min nitrogen passed through the suspension in the dark, or whether the culture was aerated in the light, the potassium efflux was practically identical (Fig. 3*a*). This indicates that once *N*-succinylperimycin has reacted it induces a permeability pathway for potassium, effective in the presence or absence of metabolic energy. On the other hand, when the suspension was well washed with nitrogen and kept in the dark before the addition of

N-succinylperimycin, no potassium leakage occurred even after a 1-h exposure to the antibiotic. The subsequent introduction of oxygen and illumination caused rapid discharge of the potassium gradient, similar to that seen when the antibiotic was added in aerobic conditions (Fig. 3b). This experiment clearly shows the requirement of metabolic energy for the induction by *N*-succinylperimycin of a potassium permeability pathway in *C. vulgaris*.

We conclude that the polyenes examined which induce irreversible membrane permeability changes in *C. vulgaris* (filipin III and nystatin A₁) interact with the membrane in a physicochemical manner. On the other hand, the compound whose lethal action can be reversed and the induced membrane damage repaired (*N*-succinylperimycin) requires metabolic energy for its interaction with the plasma membrane.

Our findings indicate that the formation of various types of membrane structures can be induced by polyene macrolides, depending on the structure of the antibiotic. Although very little is known about the structures created in biological membranes by polyene macrolides, hypothetical molecular models have suggested that bulky polyene-sterol complexes, half pores, or conductance channels can be formed on interaction of various polyene macrolides with sterol-containing lipid bilayers². It is reasonable to assume that only the formation of a highly specific membrane structure, namely, the ion-selective conductance channel, requires metabolic energy. The formation of ion-specific channels by *N*-succinylperimycin is indicated by the complete reversal of its killing action in a medium containing an isotonic concentration of potassium salts, added to prevent death through lack of potassium^{6,7}.

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Ionophore A23187 disrupts membrane structure by modifying protein-lipid interactions

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The physiological function of membrane-bound protein clearly depends on the nature of its neighbouring lipid¹. However, the question of how critical the protein is to the structure and function of membrane lipid has received much less attention. There is some evidence that lipid surrounding membrane protein, 'boundary lipid', may be more ordered than continuum lipid². Protein can also alter the enthalpy and temperature of pure lipid-phase transitions³. However, controversy surrounds the question of whether membrane proteins determine long-range aspects of lipid structure or merely perturb their local environments^{4,5}. Here, we demonstrate that a lipophilic substance, the calcium ionophore, A23187 (Lilly), dramatically disorders the lipid structure of the membrane and that this phenomenon depends on the presence of proteins.

We used the polyene fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene (DPH), to measure membrane lipid order⁶. Steady-state fluorescence polarisation was measured with an SLM model 4800 fluorescent phase modulation lifetime machine⁷. All measurements were corrected for scatter. Lifetime measurements were made with the same machine. Fluorescence spectra were obtained with a Perkin-Elmer model MPF-2a spectrophotometer. The probe was incorporated into cells (fresh splenic mouse lymphocytes, BHK or 3T3 cells grown in culture) and multilamellar lipid vesicles as previously described⁸. Vesicles were prepared from lipid extracts of cell membranes⁹ or with purified phospholipids (Sigma). Purified (Na⁺ + K⁺)ATPase (Sigma) was reconstituted into lipid vesicles by solubilising both lipid and protein at a 10:1 (w/w) ratio in 10 mM Tris, 120 mM NaCl and 5% Na deoxycholate (DOC). The DOC was removed by dialysis for 72 h. Spectral analysis revealed that about 25% of the original protein could be incorporated into the resulting vesicles. Freeze-fracture electron microscopy of the reconstituted vesicles showed many intramembranous particles.

Figure 1 shows the dramatic DPH polarisation changes in lymphocytes as a function of the concentration of A23187. They suggest marked alterations in lipid packing due to the ionophore. The *P* value change occurred within 30 s and was not affected by the addition of EGTA or alteration of the concentrations of Ca²⁺ or Mg²⁺. It was not inhibited by 10 µg ml⁻¹ of cytochalasin B, 10⁻⁶ M colchicine or 10⁻³ M NaN₃. The effect persisted despite washing and resuspending the treated cells, and was not abolished by heating the cells to 65°C for 15 min. This dose-dependent lowering of the *P* value was seen with all cell types tested. However, these polarisation changes were not found in pure lipid systems (even at 10⁻⁴ M A23187). Thus, as shown in Table 1, A23187 did not affect the *P* value of DPH-labelled liposomes. Reconstituted vesicles, on the other hand, containing protein and lipid, exhibited a diminution in DPH polarisation comparable to that of whole cell membranes.

Spectral analysis showed that there was no alteration in DPH excitation or emission spectra produced by the ionophore. Fluorescence lifetimes of DPH in cell membranes were measured. These were unaltered in the presence of the ionophore, indicating that the steady-state polarisation changes reflect true alterations in the rotational correlation times of the probe.

It surprised us that this lipophilic drug required protein to exert its 'fluidising' effect on membrane lipids. It is possible that the drug binds to proteins, decreasing the overall probe polarisation by displacing 'rigid' boundary lipid. However, extraction of membrane proteins and hence of boundary lipid results in only a 20% diminution in DPH polarisation (Table I). This is

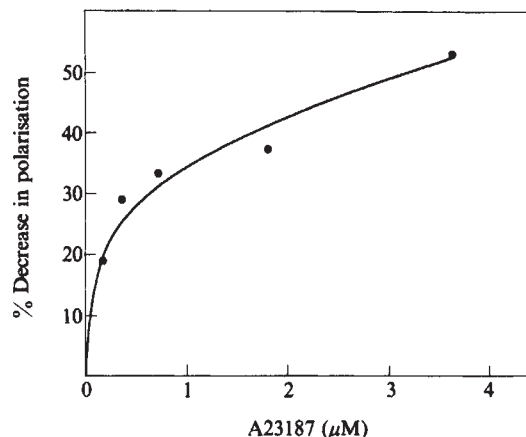


Fig. 1 Percentage decrease in polarisation of DPH as a function of the concentration of A23187 added to lymphocyte membranes. Baseline polarisation is 0.268 ± 0.012 (s.e.m.)

Table 1 The effect of A23187 on DPH polarisation

Lipid	P Value	
	Control	+A23187
Phosphatidylcholine dimyristoyl		
4°C	0.440 ± 0.006	0.438 ± 0.005 (1 µM)
23°C	0.254 ± 0.014	0.250 ± 0.011 (1 µM)
30°C	0.160 ± 0.008	0.160 ± 0.006 (1 µM)
Liposomes from:		
Lymphocytes	0.252 ± 0.012	0.250 ± 0.008 (1 µM)
BHK cells	0.268 ± 0.010	0.276 ± 0.006 (1 µM)
Liposomes reconstituted with (Na ⁺ + K ⁺)ATPase		
Lymphocytes	0.304 ± 0.018	0.220 ± 0.028 (0.72 µM)
		0.178 ± 0.040 (1.44 µM)
BHK cells	0.280 ± 0.022	0.245 ± 0.020 (0.72 µM)
		0.220 ± 0.019 (1.44 µM)

Polarisation of DPH in pure phosphatidyl choline vesicles above, at and below the phase transition is not altered by the addition of A23187. Liposomes made from lipid extracts of lymphocytes or BHK cells and tested at 23°C also seem to be unperturbed by the ionophore. Vesicles reconstituted with (Na⁺ + K⁺)ATPase show dose-dependent alterations in DPH polarisation with A23187. Values given as mean and s.e.m.

much smaller than the change induced by A23187. Thus, we consider this explanation unlikely. It is more likely that the ionophore can exert its disordering influence only when the lipids are in specific configurations. Thus, perhaps pure lipids cannot assume such structure, but the insertion of proteins

reorganises the lipids into appropriate configurations. Analogies exist: for example, chlorpromazine alters lipid structure, but only in the presence of appropriate cholesterol content¹⁰.

The nature of this special lipid configuration is unclear. However, it is unlikely to be simply the gel or liquid crystalline phases, because no effect is seen in pure phosphatidylcholine dimyristoyl vesicles either above or below the endothermic phase transition. Where does the ionophore act? One possibility is, at the lipid-protein boundary. A23187 binds to both lipids and proteins¹¹ and may well have particular affinity for the lipid-protein interface. At the lipid-protein boundary it would not act to displace boundary lipid but to alter the nature of the lipid ordering effects exerted by the membrane protein. The magnitude of our results suggests that the effect of protein on the lipid, at least by means of a perturbing molecule, can be large.

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An incipient 3₁₀ helix in Piv-Pro-Pro-Ala-NHMe as a model for peptide folding

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The molecular mechanism of helix nucleation in peptides and proteins is not yet understood and the question of whether sharp turns in the polypeptide backbone serve as nuclei for protein folding has evoked controversy^{1,2}. A recent study of the conformation of a tetrapeptide containing the stereochemically constrained residue α -aminoisobutyric acid, both in solution and the solid state, yielded a structure consisting of two consecutive β -turns, leading to an incipient 3₁₀ helical conformation^{3,4}. This led us to speculate that specific tri- and tetrapeptide sequences may indeed provide a helical twist to the amino-terminal segment of helical regions in proteins and provide a nucleation site for further propagation. The transformation from a 3₁₀ helical structure to an α -helix should be facile and requires only small changes in the ϕ and ψ conformational angles and a rearrangement of the hydrogen bonding pattern⁵. If such a mechanism is involved then it should be possible to isolate an incipient 3₁₀ helical conformation in a tripeptide amide or tetrapeptide sequence, based purely on the driving force derived from short-range interactions. We have synthesised and studied the model peptide pivaloyl-Pro-Pro-Ala-NHMe (compound I) and provide here spectroscopic evidence for a 3₁₀ helical conformation in compound I.

The choice of the pivaloyl blocking group at the amino-terminal was dictated by the necessity of maintaining the first amide bond in a *trans* configuration. While X-Pro bonds can exist in both *cis* and *trans* configurations in solution⁶, the bulky

tertiary butyl group locks the piv-Pro bond into the *trans* structure⁷. ¹³C NMR studies, of a number of pivaloyl-Pro containing peptides, show no evidence for the presence of the *cis* isomer (unpublished observations). The Pro-Pro-Ala sequence was chosen because of the strong tendency of Pro-X sequences to form β -turns⁸, a consequence of the restriction of the ϕ angle in the pyrrolidine ring to $\sim -60^\circ$. The carboxyl terminal was converted to the methylamide to provide the NH group necessary for stabilising the second β -turn by formation of an intramolecular 4 $\leftarrow$ 1 hydrogen bond.

Figure 1a and b shows the 270 MHz ¹H and 67.89 MHz ¹³C NMR spectra of I in CDCl₃. It is clear from both spectra that of the four possible isomers about the pivaloyl-Pro and Pro-Pro bonds (*trans-trans*, *trans-cis*, *cis-trans* and *cis-cis*) only one is present in solution. The chemical shifts of the C β and C γ resonances of both Pro residues (Table 1) fall within the range expected for *trans* X-Pro bonds⁹, suggesting that both the pivaloyl-Pro and Pro-Pro bonds are exclusively *trans*. The effect of changing temperature and solvent on the chemical shifts of the Ala and methylamide NH groups, are summarised in Table 1. Both amide hydrogens show a very small temperature coefficient in (CD₃)₂SO and almost no change in chemical shift on going from a poor hydrogen accepting solvent, such as CDCl₃, to a good acceptor, such as (CD₃)₂SO.

These results provide clear evidence that both NH groups in the molecule are shielded from the solvent⁹. The IR spectrum of I in dilute CHCl₃ solution shows the presence of only one N-H stretching band at 3,340 cm⁻¹, demonstrating that the NH groups are involved in the formation of intramolecular hydrogen bonds. The lack of a band at $\sim 3,430$ cm⁻¹ suggests that conformations with free NH groups are absent. Figure 2 shows a possible conformation of compound I with the Ala and methylamide NH groups involved in 4 $\leftarrow$ 1 intramolecular hydrogen bonds. While the available data only provide evidence for the participation of NH groups in hydrogen bonds, we propose the consecutive β -turn structure taking into account the known propensity of Pro residues to initiate β -turns⁸. Indeed the absence of a free NH stretching band at $\sim 3,450$ cm⁻¹ argues for

Table 1 NMR parameters for pivaloyl-Pro-Pro-Ala-NHMe

Solvent	NH chemical shifts (δ)		$\Delta\delta$ (p.p.m.) per $^{\circ}\text{C}^*$		$J_{\text{C}^{\alpha}\text{H-NH}}$ (Hz)	^{13}C chemical shifts	
	Ala	NHCH ₃	Ala	NHCH ₃		C $^{\beta}$ Pro	C $^{\gamma}$ Pro
CDCl ₃	7.37	7.01	2.5×10^{-3}	2.9×10^{-3}	9.2	27.16	24.55
(CD ₃) ₂ SO	7.39	7.07	1.5×10^{-3}	1.6×10^{-3}	8.9	29.64	26.69
						26.52	23.98
						27.07	26.05

Spectra were recorded on a Bruker WH-270 FT-NMR spectrometer at the Bangalore NMR facility. ^1H and ^{13}C chemical shifts are expressed as δ (p.p.m.) downfield from the corresponding resonances of internal tetramethylsilane.

* Temperature coefficients of NH resonances.

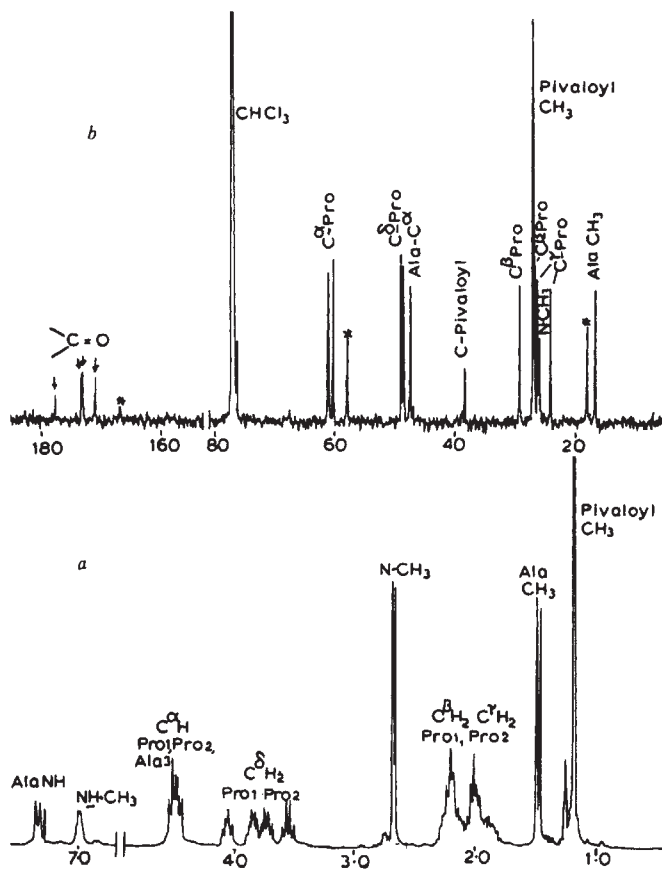


Fig. 1 a, 270 MHz ^1H NMR spectrum of compound I in CDCl₃. b, 67.89 MHz ^{13}C NMR spectrum of I in CDCl₃. Starred peaks are due to impurities in the solvent.

an almost exclusive preference for the highly folded incipient 3_{10} helical structure shown in Fig. 2. The observed $J_{\text{NH-C}^{\alpha}\text{H}}$ values of 9.2 Hz and 8.9 Hz in CDCl₃ and (CD₃)₂SO for the Ala NH are compatible with a ϕ value of $\sim -80^{\circ}$ for the Ala residue¹⁰. The presence of two Pro residues with $\phi_{\text{Pro}} \sim -60^{\circ}$ and the constraints of two intramolecular $4 \leftarrow 1$ hydrogen bonds suggested that ψ_{Pro} values¹¹ are likely to be restricted to the range -20° to -40° . These values are very close to those expected theoretically in a 3_{10} helix ($\phi \sim -60^{\circ}$, $\psi \sim -30^{\circ}$).

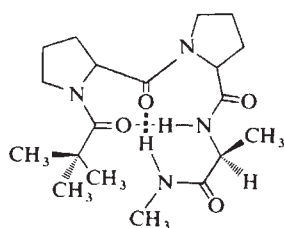


Fig. 2 Proposed 3_{10} helical conformation of compound I showing the two intramolecular $4 \leftarrow 1$ hydrogen bonds.

The importance of the methylamide NH group in stabilising this folded structure is emphasised by the fact that in the related compound pivaloyl-Pro-Pro-Ala-OCH₃, the presence of three conformers in the ratio 37:28:35 can be detected by 270 MHz ^1H NMR in CDCl₃. Further, the Ala $J_{\text{NH-C}^{\alpha}\text{H}}$ values of 7.3, 7.7 and 7.0 Hz in the three species reflect conformational averaging over the Ala N-C $^{\alpha}$ bond and may be contrasted with the J value obtained for I.

The above results provide direct evidence of a 3_{10} helical structure in a tetrapeptide containing some amino acids that occur frequently in proteins. In view of the frequent observation of 3_{10} helical segments at the ends of α -helical regions in protein crystal structures¹², it seems that consecutive β -turn structures have a definite role in giving the polypeptide chain a helical twist. In particular, peptide sequences containing a cluster of residues with a strong tendency to occur in β -turns, like Pro, Gly, Asn, Asp, Ser and Cys¹³, may have an important role in forming 3_{10} helical segments. Experimental tests of this hypothesis are under way.

We thank Ch. Pulla Rao for recording the IR spectrum and the University Grants Commission, India, for financial support.

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Corrigendum

In the letter 'Parity nonconservation and stellar collapse' by A. Vilenkin, *Nature* **280**, 569-570 it was argued that the direction dependence of the neutrino mean free time in a magnetic field $\mathbf{B}$ gives rise to a neutrino energy flux parallel to $\mathbf{B}$. However, it can be shown from the Boltzmann equation that scattering reactions ($\mathbf{p}, \mathbf{q} \rightarrow \mathbf{p}', \mathbf{q}'$) and ($\mathbf{p}', \mathbf{q}' \rightarrow \mathbf{p}, \mathbf{q}$) where $\mathbf{p}$ and $\mathbf{q}$ are electron and neutrino momenta and r is electron spin, balance in such a way that the net result is zero; that is, there is no neutrino energy flux in a magnetic field. The author thanks Ch. G. Van Weert and L. van den Horn for a discussion which helped to reveal the error.

reviews

Science policy in the developing world Ziauddin Sardar

Science, Technology and Development: Essays in Honour of A. Rahman. Edited by K.D. Sharma and M.A. Qureshi. Pp. 478. (Sterling Publishers: New Delhi, 1978.) Rs 120.

THIS anthology of papers on the themes of science policy, science and society, technology assessment, technology and underdevelopment, research management and science in India is a fitting tribute to Professor A. Rahman, "whose pioneering work", in the words of J. J. Soloman, "in science policy studies related to the problems of development has been outstanding". The idea of honouring a Third World scientist who has made a varied and sustained contribution to knowledge is indeed appropriate and there must certainly be others who would have liked to be associated with it.

Science, Technology and Development opens with a brief biographical sketch of A. Rahman. Rahman was educated at the Aligarh Muslim University and in England where he studied biochemistry under the Noble Laureate H.A. Cripps and came in contact with such scholars of the left as J.D. Bernal, Joseph Needham, J.B.S. Haldane and H. Levy. Their influence has obviously left its mark on his thinking and is responsible for his switch from biochemistry to the history and social philosophy of science. Rahman's distinguished career in the Council of Scientific and Industrial Research (CSIR) began at Regional Research Laboratory in Hyderabad immediately after his return from England. Since then he has served in many CSIR laboratories, moving, in 1963, to CSIR Headquarters in New Delhi to organise an information centre and to develop a systems approach to planning and resource allocation for the various laboratories of CSIR. Currently, he is Chief of Planning at CSIR and leads the Centre for the Study of Science, Technology and Development.

There are 26 papers in this presentation volume, inevitably of uneven quality. Some of the essays (for example, J. J. Soloman on "Science Policy and Social Objectives", E. B. Skolnikoff on "The Governability of Complexity", A.

H. Rubenstein, T. W. Schlie and A. D. Jedlicka on "Some Current Field of Research on 'Appropriate Technologies for the Less Developed Countries'" and S. R. Mikulinsky on "The Present State and Problems of the History of Natural Science") seem to be positively dated, some others (for example, Y. de Hemptine on "Integrated versus Sectoral Policies for Science and Technology", Jozef Bogner on "Culture, Science and Economy" and D. de Solla Price on "India as a Small Highly Developed Scientific Nation") are in the nature of fragments only; but, on the whole, there is enough thought provoking material here for the volume to be valuable to both the students and researchers of the social relations of science and technology.

The volume is at its strongest when dealing with science and technological policy in India. In a commanding analysis, A. K. N. Reddick argues for the generation of environmentally sound and appropriate technologies for rural development. His strategy is based on two components: forging strong links between the educational, scientific and technological institutions and the needs of the rural poor and their traditional technologies, on the one hand, and drastically weakening the reliance of these institutions on elitist demands and on institutions in the developed world. A strategy after my own heart, and one that C. Subramaniam shows so convincingly is bound to be fruitful.

Subramaniam describes a rural strategy developed for Karimnager, "a backward district of Andhra Pradesh". The work in Karimnager began with an assessment of soil, geohydrological and forest resources, an assessment of human potential, identification of new irrigation wells and a determination of fertilizer doses, and proceed to the planning of feeder roads and housing colonies based on techniques that emphasise maximum use of local material and skills. A windmill was designed and built for lifting water from the only source of drinking water in the village, carried out with the help of the National Aeronautical Laboratory, "which till then used to interest itself in

nothing less lowly than designing and testing aeronautical systems for jet aircrafts". Other projects developed for Karimnager included the collections of all waste material — human, animal and agricultural — to be processed in an inexpensive biogas plant to produce domestic fuel and fertiliser, the substitution of rubber-roller type of shellers for the more conventional energy: sheller in rice processing equipment, and improvement of various techniques from bee-keeping, making palm-candy to tanning and securing a better extraction of fibre from banana and sisal. Karimnager, K. Subramaniam argues forcefully, is a shining example of what can be achieved when scientists turn their attention to the needs of the rural poor.

India is saturated with Karimnagers and it is reasonable to assume that this success story will be repeated elsewhere. Indeed, as K. D. Sharma and M. A. Qureshi argue in their contribution, "A Framework for Alternative Technology", India needs to develop a mix of "traditional small and big technologies to achieve composite development so as to strike a balance between the internal socio-economic situation and external political stability". Although "national prestige" and "national security" figure large in their thinking, Sharma and Qureshi are really arguing for an upgrading of traditional technologies and a little more scrutiny of high technology projects. There is little doubt that India cannot tolerate, as Ward Morehouse shows in his richly detailed study of "Scuttling the Coal Gasification Project at Hyderabad: The Perils of Public Patronage in Bridging the Development Gap", a situation where its science is unwittingly pushed forward in large, prestigious undertakings. Scientists like Rahman, who turn their attention from the glare of prestige science and high technology to the plight of the rural poor and the real issues of development, deserve commendation from all who put people before glory. □

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Chemistry at the University of Edinburgh

The Playfair Collection and the Teaching of Chemistry at the University of Edinburgh, 1713-1858. By R. G. W. Anderson. Pp.175. (The Royal Scottish Museum: Edinburgh, 1978.). Paperback £4.50.

SHORTLY after Lyon Playfair's appointment in 1858 to the Edinburgh Chair of Chemistry he presented to what is now the Royal Scottish Museum 75 diverse pieces of scientific apparatus from the University Chemical Laboratory which he believed to be of historical significance. Sixty-four of these items, thought to be associated with Black, Hope and Gregory, are extant and comprise the Playfair Collection. It is an important collection, as few pre-nineteenth century chemical artefacts of known provenance survive.

Dr Anderson devotes about half of his book to discussing the provenance of the Playfair Collection as a whole and to describing fully each item, the first detailed discussion of its kind to be published. To provide a context for this catalogue the first half of the book describes the teaching of chemistry at Edinburgh from 1713 to 1858; during most of this period the University of Edinburgh was pre-eminent in Britain in the teaching of Chemistry, thus making Anderson's book of interest to both students of early scientific apparatus and historians of science.

In the catalogue section, the entry for each item consists of physical description, illustration, brief history of the development of the class of apparatus and, for some items, evidence for associating it with a particular Professor. The illustrations, both photographs and diagrams, are excellent and the brief histories are scholarly and well-referenced. The very difficult task of substantiating the association of a particular item with a particular Professor is capably done but, as is probably inevitable, the evidence is sometimes less than convincing.

The first half of the book, the author states, is not intended to be a history of the teaching of chemistry at Edinburgh or of those who taught it. This section deals primarily with their laboratories, their acquisition of apparatus and, to a certain extent, what and how they taught. In his principal aim the author succeeds admirably while supplying sufficient biographical material to make a continuous and readable account; it is particularly valuable to have hitherto unpublished information on the first holder of the Chair, Crawford, and

extensive new information on his successor, Plummer, both of whom, other than in Underwood's *Boerhaave's Men*, have received little scholarly attention in contrast to their successors, Cullen, Black, Hope and Gregory. Anderson's account is well-referenced, mainly to primary sources, and is very lucid. The locations of the various laboratories are identified with very extensive evidence. Perhaps of wider general interest are the types of apparatus acquired, the costs thereof and the sources of supply; here again the author is to be congratulated on the range and depth of his research. Less well done is the account of the teaching which, though adequate as to the method of teaching, gives little information on the content of the courses. There are a few minor errors: for example, Crawford was allotted money not "to put up his stalls" (p4) but his "stells", Old Scots for "stills"; again, Lyon Playfair was a

candidate for the Edinburgh chair in 1844 (p55) as the Town Council Minutes relating to that appointment show.

One matter of opinion on which I must express disagreement with the author is his view that *Opera Chemica* is a student notebook of Plummer's lectures. I believe it to be a set of instructions for the pharmaceutical preparations carried out in Plummer's laboratory; it is difficult to believe from Plummer's two published chemical papers that his chemical lectures were so narrow as to consist merely of pharmaceutical processes. These minor criticisms do not detract from my view that this is a splendid book which will interest not only the specialist but also the general reader and which at £4.50 is remarkable value.

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Origins uncertain

The Origin of the Solar System. Edited by S. F. Dermott. Pp. 668. (Wiley: Chichester, UK, and New York, 1978.) £28.50.

WE know and understand less about the Earth's interior than the Sun's. We know less about the origin of the planets than we do about the formation of stars and even (one may feel) of the Universe. This will be apparent to anyone who reads *The Origin of the Solar System*, the proceedings of a NATO Advanced Study Institute held at the University of Newcastle in 1976, and capably edited by S. F. Dermott.

The reason for this state of affairs is clear enough: we have only one example of a planetary system with closely measured physical properties, whereas we can observe a large number of stellar systems (and it is difficult to falsify statistically predictions about the Universe). Nevertheless, it leaves one uneasy to see a field of science so dominated by speculation, with so little hard observational foundation. For that reason one cannot escape a sense of unreality when reading this volume, which could not have been readily remedied in 1976. The discovery since then of strong candidates for pre-planetary discs around a handful of nearby stars by the Steward Observatory's infrared observing group gives some grounds for hoping that we may be able eventually to test hypotheses against planetary systems observed now to be in the process of forming. Certainly we are learning much about the Sun's birth processes — a subject not given much prominence in the present book — from recent observations of currently forming stars.

Within the book one does find various contributions which are well written and which may serve as excellent introductory reviews of specialised topics. The article by Kirsten on the use of radioactive decay for chronology using samples from the Earth, the moon and meteorites starts with an admirable discussion of principles and their application, and contains exact descriptions of a variety of experiments. One does not need to agree with the minutiae in his final cosmochronology to be impressed with the precision, and the well understood limits to that precision, of estimates such as 4.5 to 4.6×10^9 yr for the epoch of Solar System formation. A complementary paper by Larimer on meteorites in an essentially similar style is also thorough and readable. In a very different type of article Stevenson sets out clearly how global physical observations of the outer planets — their mean densities, gravitational moments and their recently measured internally generated luminosities — set constraints on their physical and chemical structures. One is then left with the problem of how the resulting abundance ratios of hydrogen to helium and to heavier elements came to deviate from their cosmic and especially their solar values. Neglecting such cosmogonic considerations we have models for the interior of Jupiter more reliable and more able to describe observable phenomena (principally its huge magnetosphere) than comparable models for the Earth.

It is on the theoretical side where credibility wears thin. Reeves acknowledges this in his introduction, where he outlines four types of theories for planetary formation, two of which were postulated in their original forms in the eighteenth century, but all of which have their contemporary protagonists.

These are the single nebula hypotheses of Kant (1755) and Laplace (1796), the accretion theory of Berkeland (1912), the exploding binary component model originally proposed by Gunn (1932) and the two-star tidal filament picture first proposed by Buffon (1745). Each has been fully articulated by modern physicists (Hoyle, for example, has contributed to all of them), who have introduced much inventive detail. The earlier chapters of this book, written by authorities such as Cameron, McCrea, Woolfson and Prentice provide well-written accounts of particular modern variants of these theories. Each is able to account, more or less, for the principal features of the Solar System: the coplanarity of planetary orbits, the contrary imbalances of mass and angular momentum between Sun and planets, the Titius-Bode orbit relationship, and planetary chemical compositions. Each such chapter is well-reasoned and persuasive, with the exception of an

irritatingly dogmatic essay by Alfvén, ostensibly critical of myth in science, but guilty of more unsupported assumption than any of the theories criticised.

Considered as a snapshot of the way the subject looked in 1976, and as a wide-ranging introduction to various aspects of the subject for students with a physics background, the book has much to recommend it. It is also a valuable case-study of the Popperian dictum that a necessary criterion for judging a scientific theory is its experimental or observational falsifiability. Although much of the theoretical work presented yields predictions which are falsifiable in principle, the authors must be aware that they are safe in practice, at any rate for the next few years. Only time and improved instrumentation will reveal the extent of their self-indulgence.

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Cancer for the layman

The Wayward Cell. Cancer: Its Origins, Nature, and Treatment. Second edition. By V. Richards. Pp. 407. (University of California Press: Berkeley, Los Angeles and London, 1978). £8.75.

The Wayward Cell is a most interesting and unique treatise on various aspects of oncological diseases, their origins, nature and clinical care. It aims to provide facts and figures about cancer which are easily understandable to the intelligent layman as well as those involved with paramedical

disciplines. However, I believe that this book will also interest and stimulate clinical oncologists, both those who are established and those in training.

The idea for the book almost certainly was born from the frustrations which clinicians not infrequently experience both with respect to their own failure to cure some tumours and with the need to provide clear concise explanations of the protean manifestations of cancer often sought by their patients.

This volume is divided into four main sections dealing with the biology, clinical aspects and treatment of cancer and some of the social problems it causes. The biology part is concerned with aspects of cell

growth and differentiation together with modern views of the importance of molecular biology in enabling us to understand how cells work.

The clinical section is perhaps the best part of the book with a fascinating and informative account of the history of cancer. The importance of exposure to environmental carcinogens in causing the numerous cancers of this day and age is dealt with logically and clearly together with a simple account of the ways in which such carcinogens might influence cell growth and function. It is perhaps a pity that this section contains no simple pathological classification of the various types of tumours to illustrate their diversity of structure and histogenesis.

The section on treatment will be of particular value to the non-medical person giving, albeit with perhaps undue emphasis on breast cancer, the background to the various therapeutic modalities in current use.

The final part of the book is called "Cancer — 1977" and is an attempt to present the current state of our knowledge, its deficiencies and the prospects for the future. This section is disappointing, being too detailed and scientific for the lay reader. Elements would have been better integrated with the other sections.

Overall, this is a good book, well written and excellently illustrated with clinical case reports to reveal the patient problems which tumours create. Very few books of this nature are available with such a wealth of information. It should interest the range of readers for which it is intended.

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Selected immunological methods

Immunological Methods. Edited by I. Lefkovits and B. Pemes. Pp. 488. (Academic, New York and London, 1979.) \$36; £23.40.

Immunological Methods is a misleading title for this volume of techniques. A non-immunologist hoping to find recipes for standard immunological methods, such as immunodiffusion in agar and immunoelectrophoresis, will be disappointed. A better title would be: *Experimental Techniques used by Scientists at the Basle Institute for Immunology*. This title, although slightly long for the spine of the book, would justify the inclusion of the chapters on nanomole peptide mapping (B.A. Moss, chapter 3), the production of sendai virus

(R. Tees, chapter 30), the running of an isotope laboratory (H.M. Pohlit *et al.*, chapter 33) and the excellent introduction to polyacrylamide slab gel electrophoresis (B. Takacs, chapter 4), all of which can hardly be described as immunological methods.

The editors have pursued a policy of allowing individual contributors freedom of style and content. This approach has resulted in a variation in depth of treatment afforded different topics and an emphasis on murine cellular immunology. For example, serological typing of human histocompatibility antigens is briefly treated in 10 pages (J.W. Stocker and D. Bernoco, chapter 14). An assay for specific alloantigen binding T cells activated in the murine mixed lymphocyte reaction is treated in detail and clarity in 17 pages (Elliott *et al.*, chapter 21).

Another problem resulting from the lack of editorial direction is the repetition of several techniques in different chapters, the most extreme example of which is the description of suspension cloning

techniques for lymphoid cells in three chapters (M. Shulman, chapter 20; N.N. Iscove and M.H. Schreier, chapter 29; G. Köhler, chapter 32).

Among the better chapters in the volume is a delightfully written account of antibody binding characteristics (S. Fazekas de Groth, chapter 1). Another bonus is the presence of techniques for making monoclonal antibodies (G. Köhler, chapter 31), although this latter chapter would have been more valuable if it had been updated to include PEG fusion techniques and extended to include different screening techniques.

This book can be commended to specialist immunologists, particularly cellular immunologists, but does not serve as an introduction to immunological techniques for the non-specialist.

Peter Goodfellow

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Arthropod phylogeny

Arthropod Phylogeny: With Special Reference to Insects. By H.B. Boudreaux. Pp. 320. (Wiley: New York and Chichester, UK, 1979.) £15.

THE past fifteen years have seen the publication of major works on arthropod phylogeny by Sharov, Hennig, Anderson and, notably, by Manton. Professor Boudreaux has now added to them a book distinguished by two guiding principles. Firstly, he believes that the Arthropoda form a single monophyletic taxon and therefore sets himself firmly against the recent tendency to regard them as three or more lineages, each independently evolved from primitive lobopod ancestors. Secondly, as an advocate of Hennig's methods of phylogenetic reconstruction, he proceeds by defining monophyletic sister-groups strictly through their common specialisations and abandoning, without much hesitation, the paraphyletic and polyphyletic taxa of the past.

After treating the Onychophora as a phylum separate from but related to the Arthropoda, Boudreaux devotes almost a third of his book to the affinities of the arthropod classes and the remaining two-thirds to the mutual relationships of the insect orders. The great diversity of the phylum, the volume of factual material, and the closely reasoned interpretations defy a brief summary, but the book has the great merit of presenting explicitly the foundations (mainly more or less familiar morphological data from living forms) on which the inevitably somewhat speculative inferences are based. Essentially the author recognises two main branches in arthropod evolution: a Cheliceromorph line, with the Pycnogonids, Xiphosurids, Eurypterids and Arachnids as its major subdivisions, and a more varied Gnathomorph line. The latter includes the Trilobites and the Mandibulata which (*pace* Manton) comprises the Crustacea, Myriapoda and the monophyletic Insecta.

To express his phylogenetic views in taxonomic terms, Boudreaux does not flinch from introducing, between the class Insecta and its constituent orders, no fewer than thirteen different taxonomic categories and the appropriate taxa, each duly named and defined. Within this rather cumbersome framework the evolutionary affinities of the orders are discussed in ways that are sometimes widely acceptable, sometimes more controversial. The Apterygota, Palaeoptera and Exopterygota are regarded as paraphyletic and discarded, although the Entognathan apterygotes are treated as a natural group. The order Mallophaga is restricted to the Amblycera, while a sister-group, the Anoplura, is redefined to accommodate

the Ischnocera, Rhynchophthirina and Siphunculata. The Plecoptera, Dermaptera and Embioptera, all with notoriously obscure affinities, are hardly more convincingly placed than in alternative classifications, and similar doubts might also apply to the Thysanoptera, Strepsiptera and Siphonaptera. The Sialoidea, Raphidioidea and Plannipennian Neuroptera all receive separate ordinal status, but the Boreidae and Micropterigidae are denied it.

In many respects the resulting scheme for the insects is not unlike those proposed by Hennig or Kristensen. What must strike a more sceptical reader, however, is the tantalising scarcity of really crucial evidence and fully convincing arguments.

Despite his confident style the author does, in fact, admit the tentative nature of many phylogenetic hypotheses. Their importance lies less, perhaps, in the permanent value of the conclusions reached than in their immediate heuristic value. In this sense the views and arguments of Boudreaux and those he represents should be welcomed: they will, along with the theories of their opponents, promote the study of Arthropod morphology, development and classification, and so, one hopes, make possible one day a more widely agreed phylogeny of the Arthropoda.

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Semiconductor devices

Semiconductors and Electronic Devices. By A. Barl-Lev. Pp. 381. (Prentice-Hall International: London and Englewood Cliffs, New Jersey, 1979.) Hardback £11.95, \$22.95; paperback £8.95, \$18.95.

THIS medium-sized book written by an electrical engineer for electrical engineers embarks on the ambitious task of introducing the reader to the physical principles of semiconductor materials and leading him on to a more detailed discussion of various aspects of junction and, to a lesser extent applications of semiconductor devices. In a sense, the title is slightly misleading, implying that devices other than semiconductor ones are being treated while, in fact, the discussion of vacuum devices is minimal.

The discussion of the physical and technological principles relating to semiconductor materials covers 114 pages in 8 chapters and is necessarily sketchy, as the author admits in the preface. However, I accept that the treatment is adequate for an electrical engineering student wishing to concentrate on the practical aspects of device performance.

The next three chapters are devoted to a discussion of various aspects of junction diodes, beginning with their static characteristics and including a description of varactor diodes. The optoelectronic devices mentioned include the PIN detectors, solar cells, light emitting diodes, optic couplers and even injection lasers — the treatment here is possibly at its most sketchy in view of the different physical background required for full understanding. A more thorough chapter on the dynamic response gives a discussion of diode switching.

The treatment of transistors begins interestingly and unconventionally with a discussion of field effect transistors both of the junction and of the insulated gate

types, with a brief reference to integrated circuit design. This is followed by a chapter on the amplifying stage, transistor model and the equivalent circuit which is based on the characteristics of the MOS transistor but includes mention of vacuum device and bipolar transistor characteristics, neither of which had yet been introduced. The discussion of bipolar transistors starts with the large signal model and maximum ratings and only then proceeds to small-signal low- and high-frequency models. This is a sensible arrangement and these two chapters show a rather deeper level of treatment than most of the others. One chapter deals with an introduction to integrated circuits, both bipolar and MOS, to charge-coupled devices and integrated injection logic.

The chapter on microwave devices describes very briefly the relevant constructions of the bipolar and junction FETs, gives a very cursory discussion of varactor, step-recovery and IMPATT diodes, and finishes with the transferred electron devices. The main body of the text is completed with a chapter on silicon-controlled rectifiers and triacs. There are three appendices on two-port representations, thermionic emission and vacuum tubes — the last two somewhat incongruous in the present context.

On the whole, as an introductory text for electrical engineers this is a good and interesting book. It mentions, if only briefly, most of the recent developments in semiconductor devices that the student is likely to come across. There are useful Problems at the end of each chapter. My general criticism of this strongly device-oriented text is the relative paucity of actual device characteristics — the student would have benefitted a great deal from being able to see more performance data. Also, the list of 22 references for further reading is not adequate to guide a reader who wishes to follow up specialised topics.

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